

Fig. 1.1

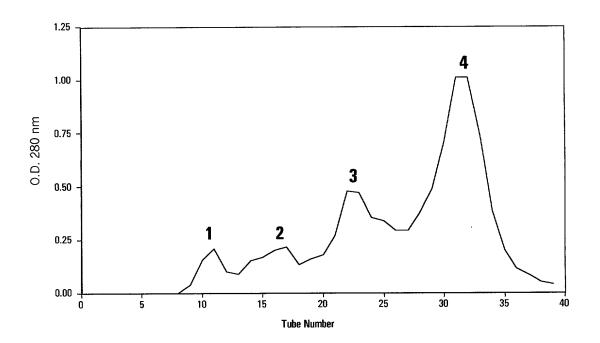
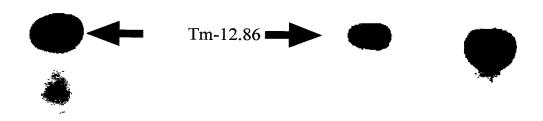


Fig. 1.2

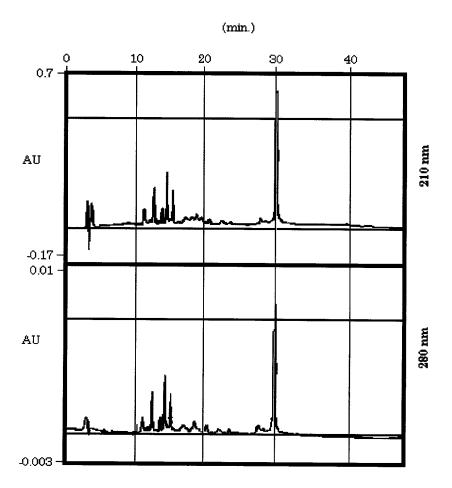


12.5

25

Fig. 1.3

Fig. 1.4



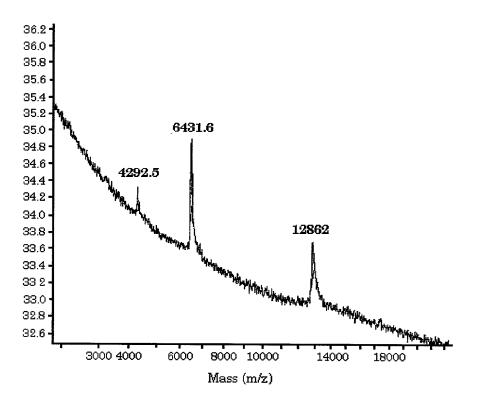
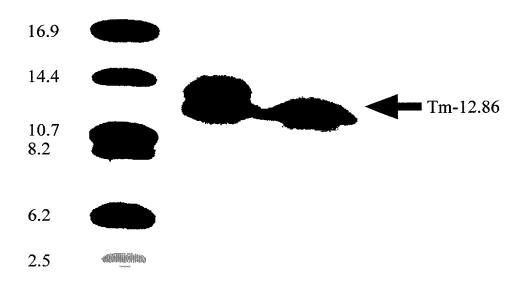
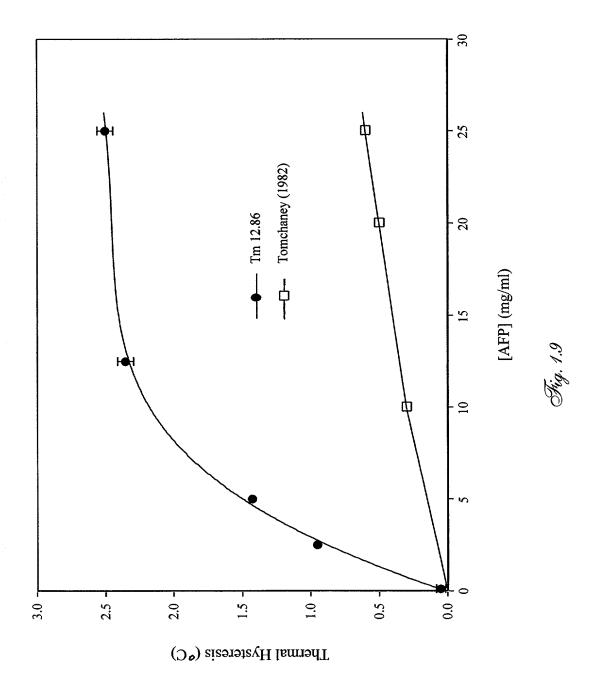


Fig. 1.6



w w/o

Fig. 1.7



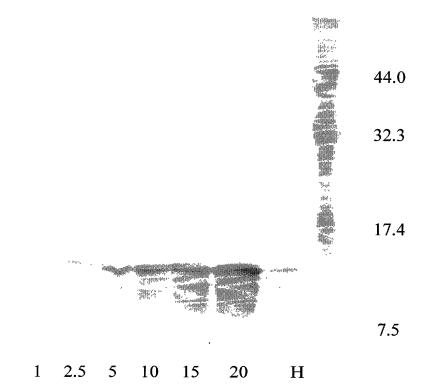
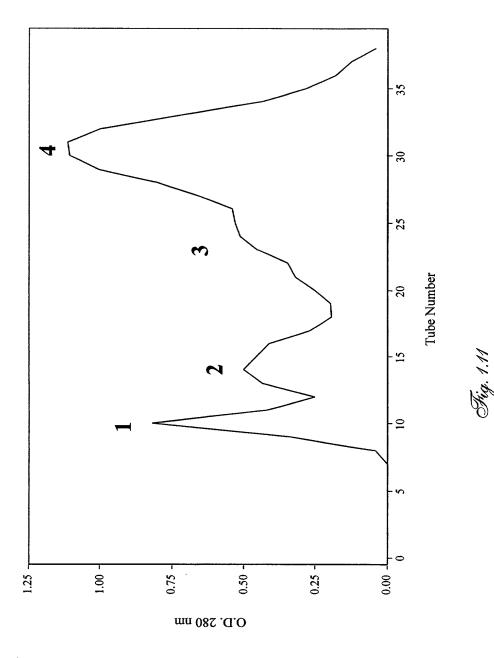


Fig. 1.10



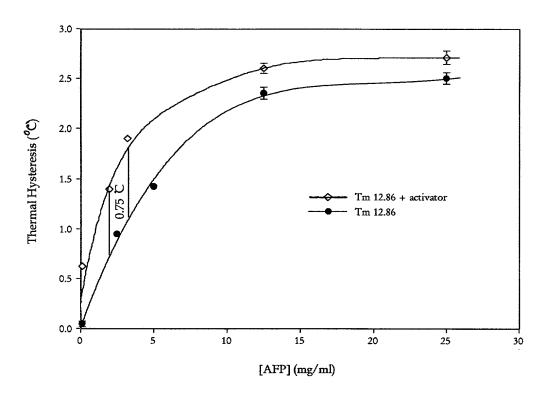


Fig. 1.12

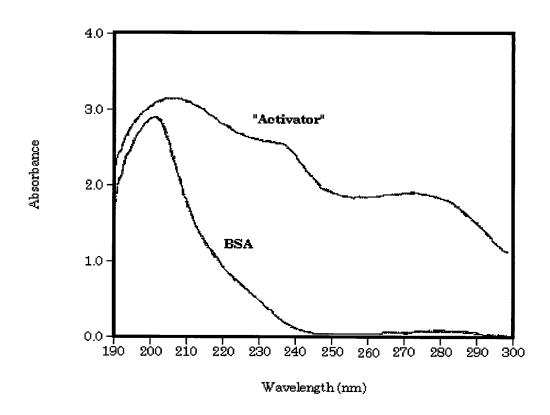


Fig. 1.13

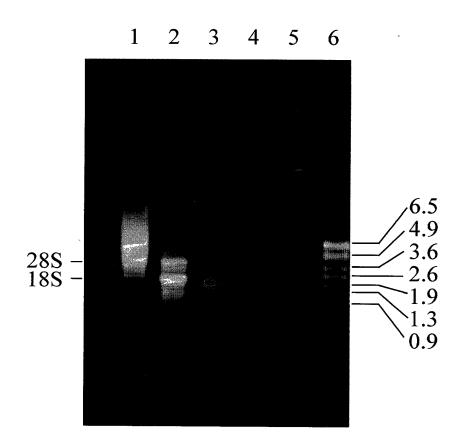


Fig. 2.0

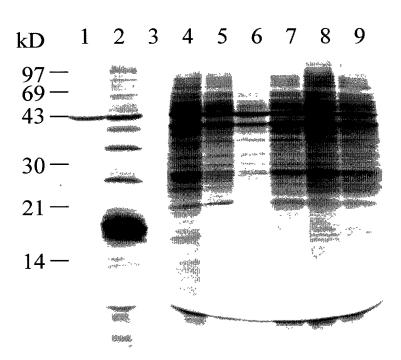


Fig. 2.1

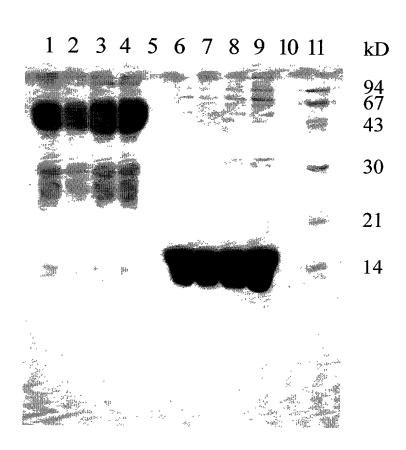


Fig. 2.2

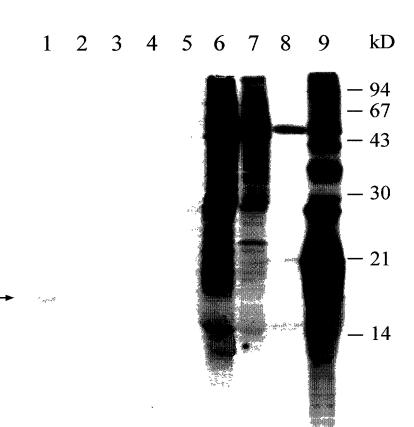
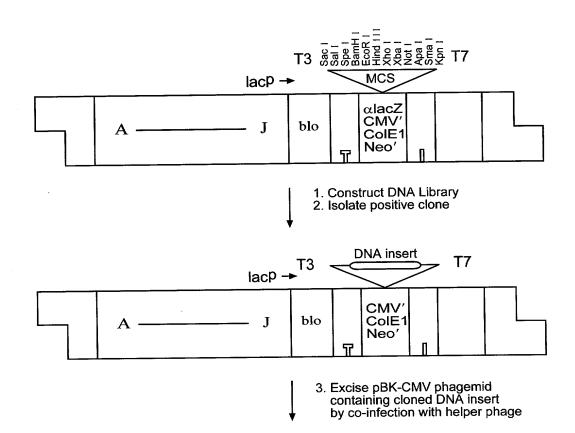


Fig. 2.3



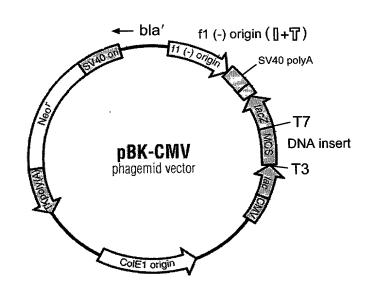
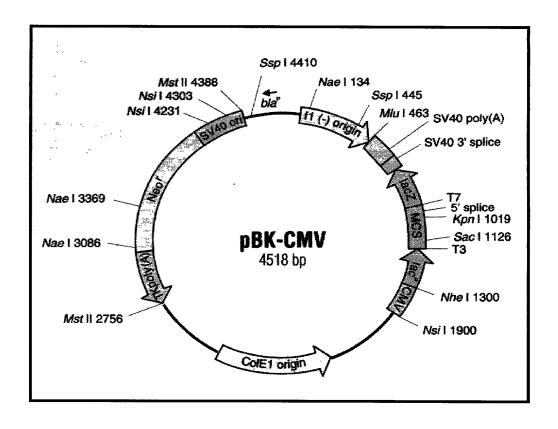


Fig. 2.4a





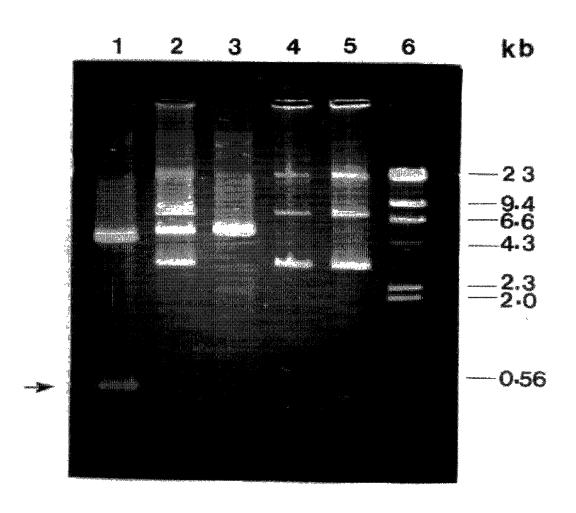


Fig. 2.5

DNA sequence of Tm 13.17 cDNA clone

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	a			C	:															
	m	ı		C	•															
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241	ACGC	CGG	TCI	'GGC	CAC	:GG#	ATC	:GGG	AGA	GGT	'GG'I	'GGT	'CGA							
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537	AGAG	TAT	TCI	AG	\GC(GCC	CGCC	3GGC	CCA	ATCG	TT	TCC	ACC	C						

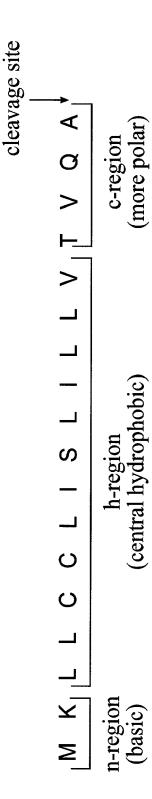


Fig. 2.66

A. Mature Tm 13.17 amino acid residure

- 1 LTEAQIEKLN KISKKCQNES GVSQEIITKA RNGDWEDDPK LKRQVFCVAR
- 51 NAGLATESGE VVVDVLREKV RKVTDNDEET EKIINKCAVK RDTVEETVFN
- 101 TFKCVMKNKP KFSPVD

B. Summary of the composition analysis for the mature Tm 13.17 sequence:

Residue	Number	Mole Percent
A = Ala B = Asx C = Cys D = Asp E = Glu F = Phe G = Gly H = His I = Ile K = Lys L = Leu M = Met N = Asn P = Pro Q = Gln R = Arg	Number 6 0 4 8 13 4 4 0 6 16 5 1 8 3 4 6 5 8	5.172 0.000 3.448 6.897 11.207 3.448 3.448 0.000 5.172 13.793 4.310 0.862 6.897 2.586 3.448 5.172 4.310 6.897 12.069
S = Ser T = Thr V =Val W = Trp Y = Tyr Z = Glx	1 0 0	0.862 0.000 0.000

Molecular weight = 13171.96; Residues = 116; Average Residue Weight = 113.551 Charge = 1; Isoelectric point = 7.74.

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A G T G G A T C C A A G A A T T C G G C A C G A G A C T A C T A A G A T G A A
                                                                                                                                                                                                                 Tn 13.17
                                                                                                                                                                                                                 B 1
               OTTOCTCTOTTOTCTANTCTCCCTCATTCTGTTOOTCACA
                                                                                                                                                                                                                  Tn 13.17
                                                                           C T T A C T T C T C T C A T T C T B T T O G T C C A
                                                                                                                                                                                                                 B 1
             Tn 13, 12
                                                                                                                                                                                                                 R 1
             R G R T C R G C R A R R R T G T C R A R R T G R A R G T G G R G T G T C C C R R R R C T G G R G T G T C C C R
                                                                                                                                                                                                                  Tn 13.17
                                                                                                                                                                                                                  8 1
            RORGATCATARCCARAGCTCOCARCOGTGACTGGGAGGAC
                                                                                                                                                                                                                  Tn 13, 17
                                                                                                                                                                                                                  8 1
              D A T C C T A A A C T O A A A C O C C A A O T T T T T T C C O T O G C C A O O A
                                                                                                                                                                                                                  Tn 13, 17
              O ACCCCA A A C TO A A A A TOC A A C T T C T T TO C A T T T T C A A O O
                                                                                                                                                                                                                  8 1
              A C O C C O O T C T O O C C A C O O A A T C O O O A O A O B T O O T O G T C O A C A C T C O A A A T C O O C A C A A A T T O A C O C C G A
                                                                                                                                                                                                                  Tm 13.17
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             C G T G T T O A G O O A A G O T G A O O A A G O T C A C T G A C A A C G A C C A C G A C A A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G A T O A C G 
                                                                                                                                                                                                                  Tn 13.17
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             GRAGAARC TO A GRAAA TCAT CAATAAG TG COCCO TCAA GA
GAAGAAG COAGAAATT OT COAGAAG TO CACGG TGAC TG
321
                                                                                                                                                                                                                  Tn 13.17
361
               CACATACTOT TO A A CACAC TO T TC A A T A CT T TC A A A T O
                                                                                                                                                                                                                  Tm 13.17
308
               A A C A C A C T C C O O A A O A T A C G C C A T T T O A A O T T A C C A A A T G
                                                                                                                                                                                                                  B 1
401
               T O T C A T O A A A A A C C A A O C C A A A O T T C T C A C C A G T T G A T T O A
                                                                                                                                                                                                                  Tm 13.17
              T O T A T T O A A G G A C A A O C C C A A T T T C T
                                                                                                                                                                                      TTOC
                                                                                                                                                                                                                  B 1
             R C C R C C A C T A C T A G A T G G T T C A A A T C O T G T C C T T T A C

O A C T A T T T G T C T C A A A T C O T G T C C T T T G T
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388
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428
               0 C A C A A
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Tm 13.17	3	EAQIEKLNKISKKCQNESGVSQEIITKARNGDWEDDPKLKRQVFCVARNA	52
AFP-3	1	.	50
Tm 13.17	53	GLATESGEVVVDVLREKVRKVTDNDEETEKIINKCAVKRDTVEETVFNTF	102
AFP-3	51	: . : ::. .:. ::. .::. : .: : GFIDASGEFQLDHIKTKFKENSEHPEKVDDLVAKCAVKKDTPQHSSADFF	100
Tm 13.17	103	KCVMKNKP 110	*
AFP-3	101	. :. KCVHDNRS 108	

Percent identity: 39.8 (identical amino acids; Percent similarity: 58.3 (identical amino acids plus conservative amino acids).

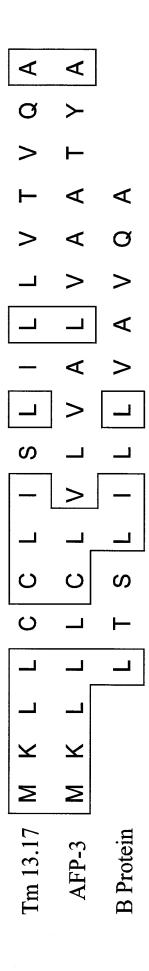
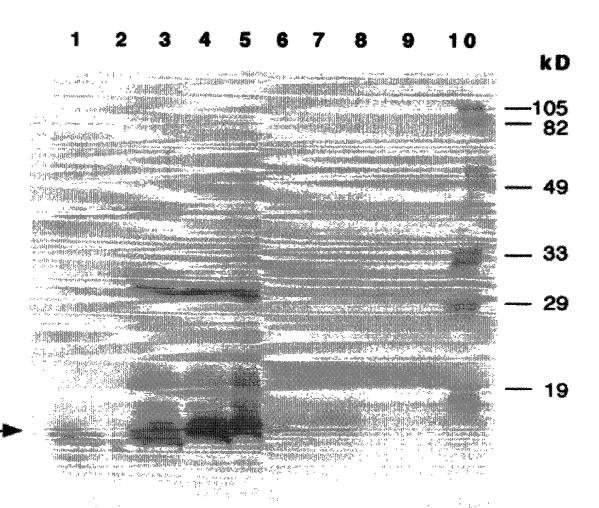
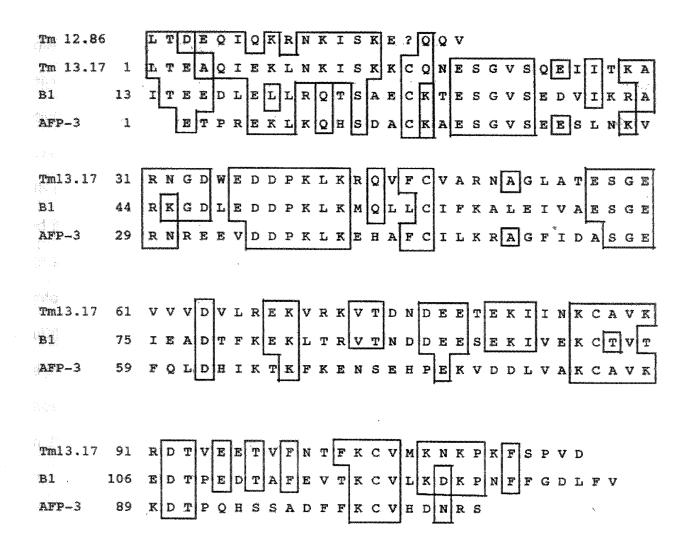


Fig. 2.9

Tm 13.17 NH2-L T E A Q I E K L N K I S K K C Q N E Tm 12.86 NH2-L T D E Q I Q K R N K I S K E ? Q Q V

Fig 2.10





1	GC	à C	; A	C	G	A	G	C A	Ą A	A	A	A M	T		A.A K	۸ <i>ا</i>	L	; T	С —	F.	T (C I	г т 	G	T C	G	C.	T 7 F	T T	A	C	G	T F	T	C (a C	- C	GCC A_
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182	C (P	c) <i> </i> }		. Α	A M		G	A A K	A C	à A K	A	G	C	A (٥ (۱	à T /	C	C	T	c T	T (3 C	F	T	С	T s	C	a A K	A	G	A	A	A :	A Ć T	T	G	G A
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271	A A					A			C # H	١,	V	iΤ	G	G A	C	0 /	4 G 3	à C	G D	A	C	G/ E	A A	G E	Α	G	Gʻ V	T	G G	i A	C	A K	A	G.	A 7 I	C	G V	TG
316	C A	A (. G	T		C	G7 V	Γ(3 G V	i T	С	A K	A (G/	A <i>A</i>	G	G	C	C <u>4</u>	A (C A	C	С	A	G. E	A (3 G	A	À	A T	С	G	G (T	T	A T
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poly (A) tail

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271	A A K		C		G .		A	G (C / H	A T	· G	T	G	G A	С	C	A (G C	G	i A	С	G E	Α.	A i	G A E	. Α	G V	Τ (G (G A D	C	: A K	A	G	A	Т (C G V	a T /	G
316	C A Q		A K			T C	G	C	G T V	۲ (G V	T	С	A K	A	G	A A	A C	à G A	à C	С	A T	C	A	C C	: A	G E	A	G	G A E	A	A T	C	G	G A	C.	T T Y	Г А ′	т
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406	A T	τ	G D	A	Т	Ţ	A	A	т .	т (3 T	т	Т	Т	G	Т	A '	TI	Т	· G	A	С	T	G.	A A	ιT	Т	T	T									3 G gna	
451	A C	Т	Α	Т	С	G	Т	T	Α.	т (a A	ιA	Α	Α	A	A	A	A A	\ A	\ A	Α.	Α	A	A	A A	λA				po	1 1 9	au	(CI)	ıyı	au	VII	. 31	₽ ₁₁₅	.,

poly (A) tail

Start

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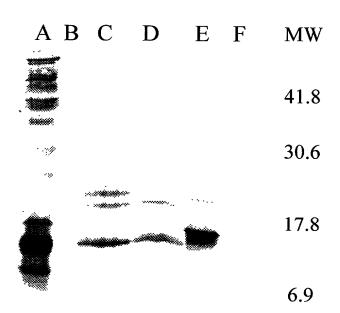
Predicted Amino Acid

Composition of 2-2 and 2-3

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

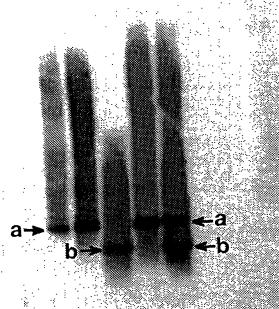
Whole Protein Composition Analysis

	Number	% by	% by
Amino Acid(s)	count	weight	frequency
Charged (RKHYCDE)	48	47.19	41.74
Acidic (DE)	20	18.90	17.39
Basic (KR)	20	20.40	17.39
Polar (NCQSTY)	30	25.35	26.09
Hydrophobic (AILFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
l lie	6	5.29	5.22
. K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	. 2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.08	7.83
V Val	14	10.80	12.17
WTrp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00





1 2 3 4 5



577 bp 483 bp

Fig. 4.0

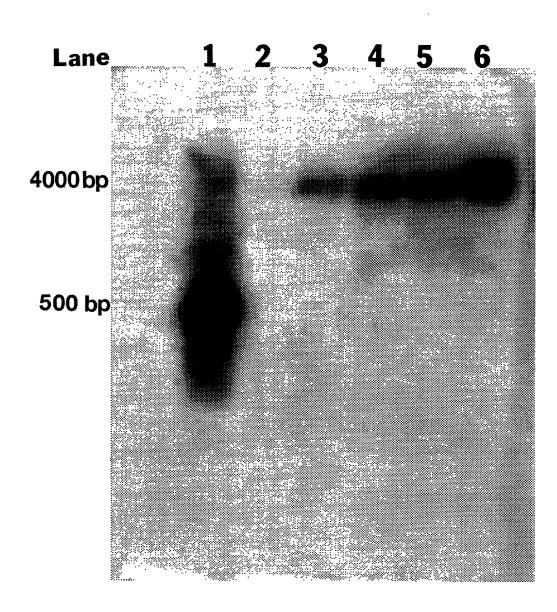
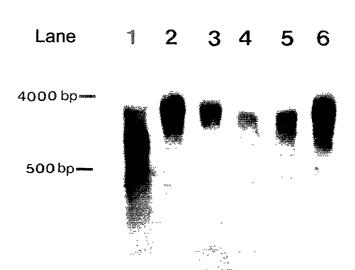
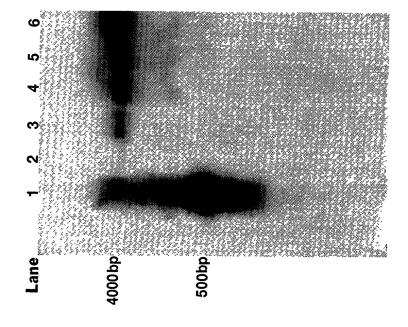
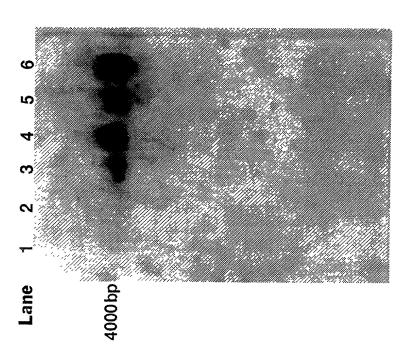


Fig. 4.1







Lane 1 2 3 4 5 6



Lane 1 2 3 4 5

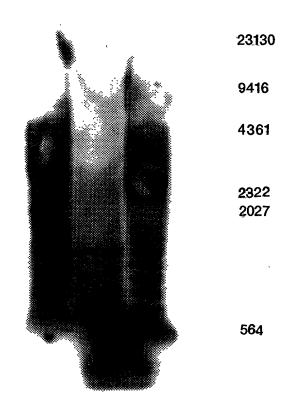


Fig. 4.5.

Tm 13.17 cDNA

1	AGTO	GAT	CCA	AAG	AAT	rrco	igc#	CGA	\GA(CTAC	TAA	GAT	GAA	GTI	GC1	CTG	TTG	TCT	AAT	CT
												M	K	L	L	C	С	<u>L</u>	<u>I</u>	S
61	CCCI	CAT	TCT	'GTT	GG1	CAC	'AGI	TCP	\GG(cca	rGAC	CGA	GGC	ACA	LAAT	TGA	GAA	ACT	GAA	CA
	<u>L</u>	<u>I</u>	<u>L</u>	L	V	T	V	Q	<u>_A</u>	♣ L	T Forw	E ard I	A Prime	Q er	I	E	K	L	N	K
121	AGAT	CAG	CAA	AAA	ATC	TCA	AAA	TGA	AA(FIG(AGT	GTC	GCA	AGA	GAT	'CAT	AAC	CAA	AGC	TC
	I	s	K	K	C	Q	N	E	S	G	V	S	Q	E	I	I	T	K	Ą	R
181	GCAA	CGG	TGA	CTG	GG#	LGGA	CGA	TCC	TAI	AC!	rgaa	ACG	CCA	AGT	TTI	TTG	CGT	GGC	CAG	GA
	N	G	Đ	W	E	D	D	P	K	L	K	R	Q	V	F	C	٧	A	R	N
241	ACGC	:CGG	TCT	'GGC	CAC	:GGA	ATC	:GGG	AG	\GG!	rggt	GGT	CGA	CGI	GTT	'GAG	GGA	GAA	GGT	GA
	A	G	L	A	T	E	s	G	E	V	V	V	D	V	L	R	E	K	V	R
301	GGAA	GGT	CAC	TGA	CAA	CGA	CGA	AGA	AAC	TGI	GAA	AAT	CAT	CAA	TAA	GTG	CGC	CGT	CAA	GA
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	D	T	V	E	E	T	V	F	N	T	F	K	C	V	M	K	N	K	P	K
421	AGTT	CTC	ACC	agt	TGA	TTG	AAC	CAC	CAC	GAC	TAG	TAG	ATG	GTT	CAA	ATG	GTG	TGC	TTT	AÇ

- 537 AGAGTATTCTAGAGCGGCCGCGGGCCCATCGTTTTCCACCC

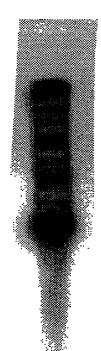
Forward Primer

2-2	LTDEQIQKRNKI SKECQQVS <mark>GVSQE</mark> TI DKVRTGVLV
Tm 13.17	LTEAQIEKLNKI SKKCQNES GVSQEI I TKARNGDWE
B2	LTEEDLQLLRQTSAECKTES GASEAVI KKARKGDLE
AFP-3	ETPREKLKQHSDACKAES GVSEESLNKVRNREEV
2-2	DDPKMKKHVLCFSKKTGVATEAGDTNVEVLKAKLKH
Tm 13.17	DDPKLKRQVFCVARNAGLATESGEVVVDVLREKVRK
B2	DDPKLKMQLLC! FKALEI VAESGEI EADTFKEKLTR
AFP-3	DDPKLKEHAFCI LKRAGFI DASGEFQLDHI KTKFKE
2-2 Tm 13.17 B2 AFP-3	Reverse Primer VAS DEEVDKI VQKCVVKK ATPEET AYDTFKCI YDS VTDNDEETEKII NKCAVKR DTVEET VFNTFKCVMKN VTNDDEESEKI VEKCTVTE DTPEDT AFEVTKCVLKD NSEHPEKVDDL VAKCAVKK DTPQHS SADFFKCVHDN
2-2	KPDFSPID
Tm 13.17	KPKFSPVD
B2	KPNFFGDLFV
AFP-3	RS

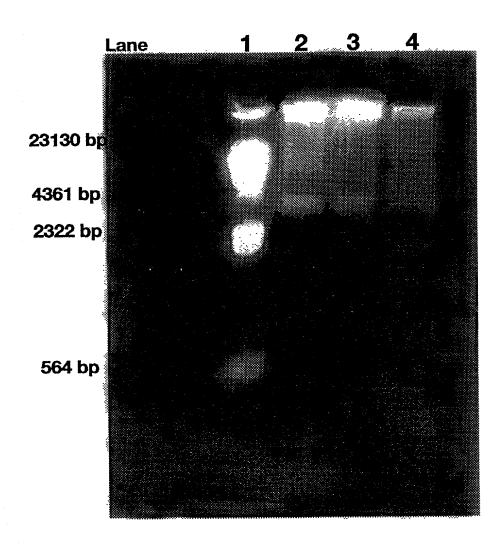
Fig. 4.6b

	percen	it % c	ompositi	ion	_
Primer	Α	С	G	Т	MeltingTemperature(^O C)
Forward	28.6	14.3	42.9	14.3	44.0
Reverse	25.0	31.3	6.3	37.5	44.0

Fig. 4.6c



3600 bp



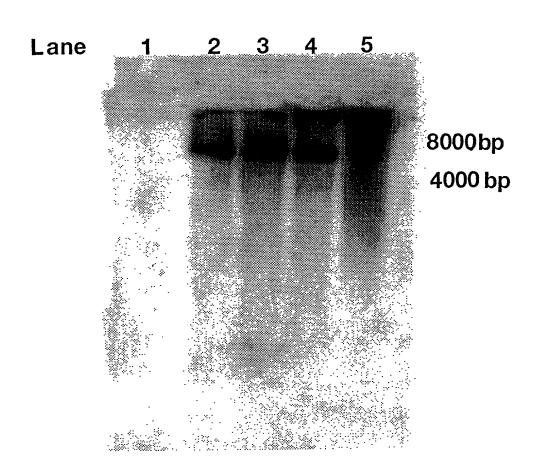


Fig. 4.9

GGCACGAGCAAAAAACTCCTCTTGTGCTTTGCTTTCGCCGCC M K L L C F A F A A ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA AGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGTGTCCRNKISKECQQVSGVS 92 137 CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGAPKMKKHVLCFSKKTG 182 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCCVA TEAGDTNVEVLKA 226 AAGCTGAAGCATGTGGCCAGCGACGAAGATGGTGK L K H V A S D E E V D K I V 271 CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT Q K C V V K K A T P E E T A Y GACACCTTCAAGGTTATTTACGACAGTAAACCTGATTTCTCTCCTD T F K V I Y D S K P D F S P 361 ATT GATTAATT GTTTTT GTATTT GACT GAATTTT GACAATAAA GGT 406 polyadenylation signal 451 ACTATCGTTATGTAAAAAAAAAAAAAAAAA

poly (A) tail

Predicted Amino Acid

Composition of 3-4

Molecular Weight	12839.70 m.w.
Length	115
1 microgram =	77.883 pMoles
Molar Extinction coefficient	2920±5%
1 A(280) =	4.40 mg/ml
Isoelectric Point	7.14
Charge at pH 7	0.16

Whole	Protein	Composition	Analysis
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Whole Fiotem Compositi	Number	% by	% by
Amino Acid(s)	count	weight	frequency
Charged (RKHYCDE)	47	46.41	40.87
Acidic (DE)	20	18.91	17.39
Basic (KR)	20	20.41	17.39
Polar (NCQSTY)	29	24.55	25.22
Hydrophobic (AILFWV)	35	28.04	30.43
A Ala	6	3.32	5.22
C Cys	3	2.41	2.61
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
l lle	6	5.29	5.22
KLys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gin	6	5.99	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.09	7.83
V Val	15	11.58	13.04
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

GGCACGAGCAAAA<mark>ATG</mark>AAACTCCTCTTGTGCTTTGCTTTCGCCGCC M K L L C F A F A A ATCGTCATCGGAGCTCAGGCTCTCACCGATGAACAGATACAGAAA IVIGAQAALT DEQIQK 47 AGGAACAAGATCAGCAAAGAATGCCAGCAGGAGTCCGGAGTGTCCRNKISKECQESGVS 92 CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT Q E T I D K V R T G V L V D D 137 CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAGAACTGGA P K M K K H V L C F S K R T G 182 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCCVA TEA G D T N V E V L K A 226 AAGCTGAAGCATGTGGCCAGCGACGAAGATGGACAAGATCGTGK L K H V A S D E E V D K I V CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT Q K C V V K K A T P E E T A Y 316 GACACCTTCAAGTGTATTTACGACAGTAAACCTGATTTCTCTCCTD T F K V I Y D S K P D F S P 361 ATTGATTAATTGTTTTTTTTGACTGAATTTTGACAATAAAGGT 406 polyadenylation signal ACTATCGTTATGAAAAAAAAAAAAAAAAAAA 451

poly (A) tail

Fig. 4.11a

Predicted Amino Acid

Composition of 3-9

Analysis	Whole Protein
Molecular Weight	12871.80 m.w.
Length	115
1 microgram =	77.689 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.23 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

	Number	% by	% by
Amino Acid(s)	count	weight	frequency
Charged (RKHYCDE)	48	47.31	41.74
Acidic (DE)	20	18.86	17.39
Basic (KR)	20	20.57	17.39
Polar (NCQSTY)	30	25.29	26.09
Hydrophobic (AILFWV)	34	27.20	29.57
A Ala	6	3.31	5.22
C Cys	4	3.21	3.48
D Asp	11	9.84	9.57
E Glu	` 9	9.03	7.83
F Phe	3	3.43	2.61
G Gly	4	1.77	3.48
H His	2	2.13	1.74
I lle	6	5.28	5.22
K Lys	17	16.93	14.78
LLeu	5	4.40	4.35
M Met	1	1.02	0.87
N Asn	2	1.77	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.97	5.22
R Arg	3	3.64	2.61
S Ser	7	4.74	6.09
T Thr	9	7.07	7.83
V Val	14	10.78	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

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Fig. 4.12a

poly (A) tail

Predicted Amino Acid

Composition of 7-5

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Whole Florein Compositi	Number	% by	% by
Amino Acid(s)	count	weight	frequency
Charged (RKHYCDE)	48	47.19	41.74
Acidic (DE)	20	18.90	17.39
Basic (KR)	20	20.40	17.39
Polar (NCQSTY)	30	25.35	26.09
Hydrophobic (AILFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
l lie	6	5.29	5.22
KLys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.08	7.83
∨ Val	14	10.80	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

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	(kDa)	(#)																				
Tm 12.86	12.86	117	3.2	3.0	3.4	4.4	8.5	2.0	4.4	28.9	3.1	3.9 3.8 3.2	3.8	3.2	ND	10.7	ND 10.7 15.0 3.6	3.6	14.9 6.8	8.9	6.3	57.3
Tm 13.17	13.17	116	3.13	2.21	4.47	5.16	10.5	1.0	4.3	29.11	1.73	1.73 3.24 0 0	0		1.41 0 0	0	0	7.12	15.6 3.31		6.14	32.14
2-2	12.84	12.84 115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	1.78 3.32 2.54 2.14 0		0	0	2.43	18.0 4.75	4.75	7.08	32.23
2-3	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	1.78 3.32 2.54 2.14 0		0	0	2.43	18.0 4	4.75	7.08	32.23
3-4	12.84	115	2.41	3.02	3.44	5.29	11.6	1.02	4.41	28.04	1.78	3.32	2.54	1.78 3.32 2.54 2.14 0	0	0	0	2.43	18.0 4.75		7.09	32.24
3-9	12.87	115	3.21	3.02	3.43	5.28	10.8	1.02	4.40	27.20	1.77	3.31	2.54	1.77 3.31 2.54 2.13 0		0	0	3.64	16.9 4.74	4.74	7.07	32.38
7-5	12.84	115	12.84 115 3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	1.78 3.32 2.54 2.14 0 0 0	0	0	2.43 18.0 4.75	18.0	4.75	7.08 32.23	32.23

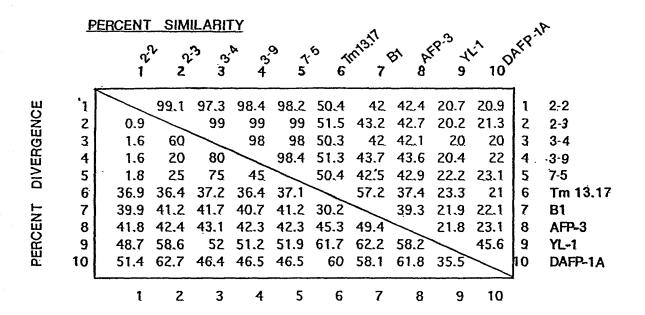
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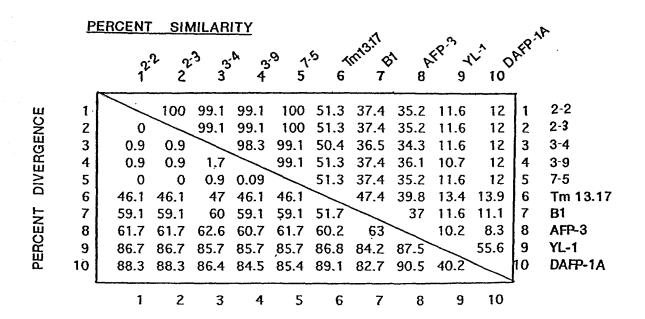
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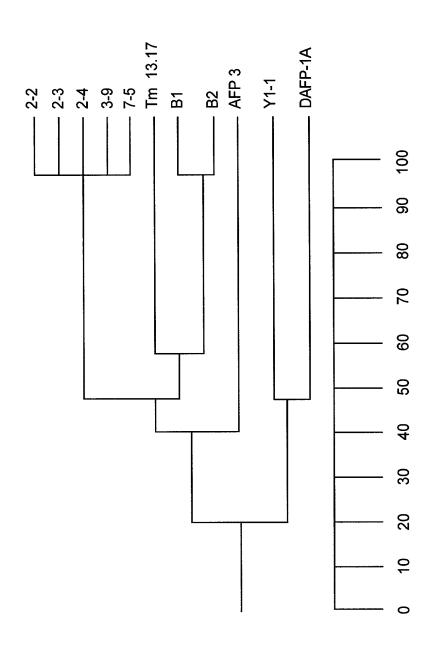
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NUCLEOTIDE SEQUENCES



AMINO ACID SEQUENCES





% Nucleic Acid Identity

Fig. 4.20

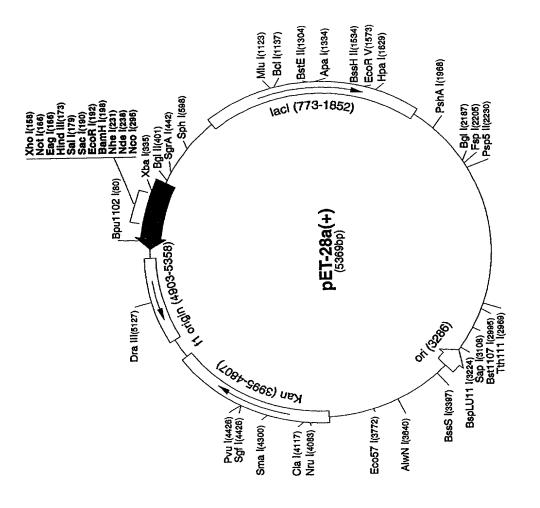
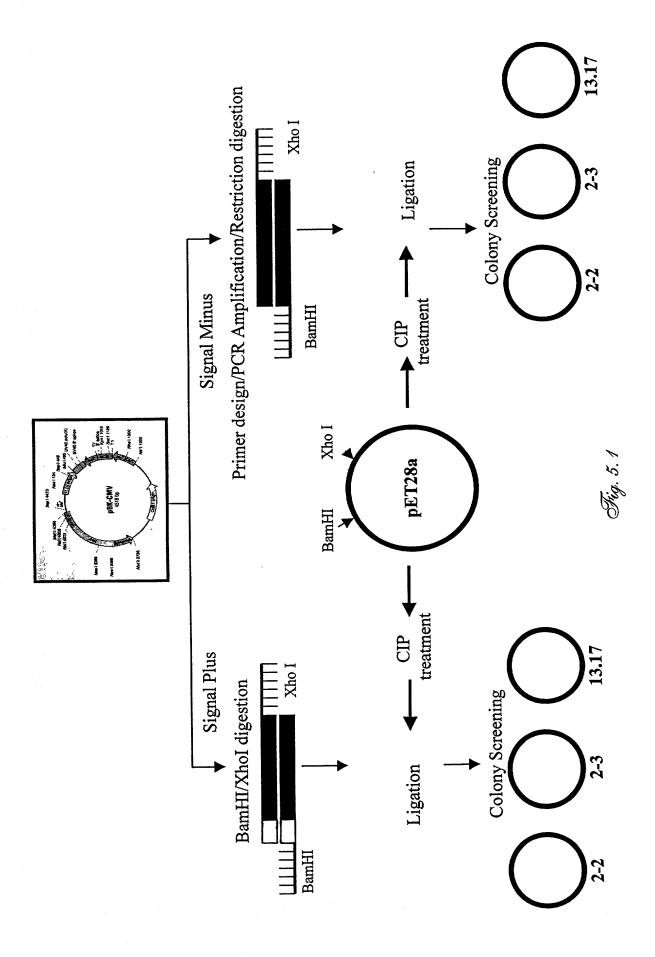
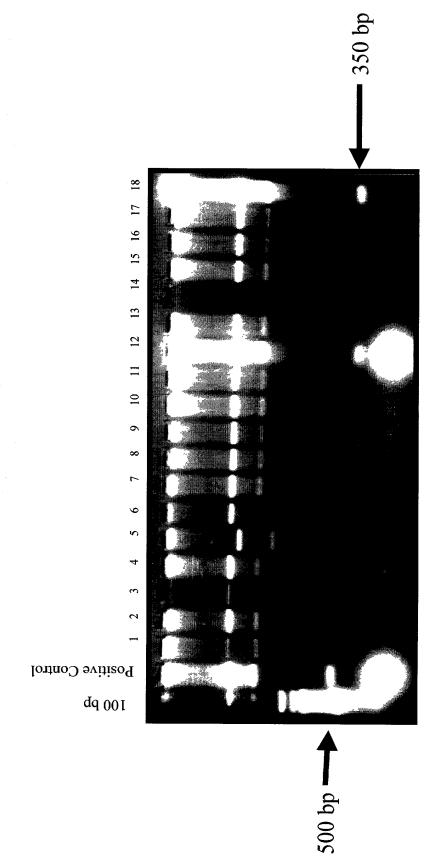
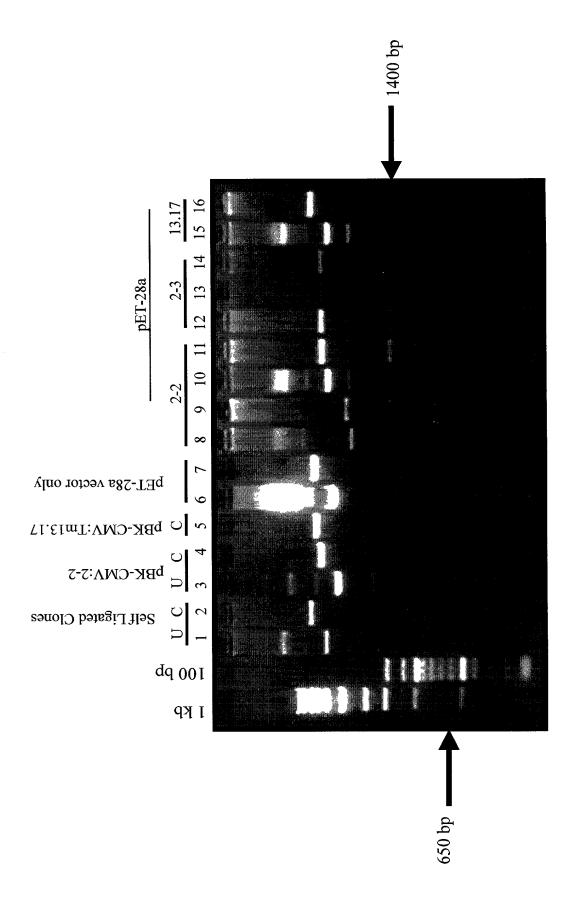
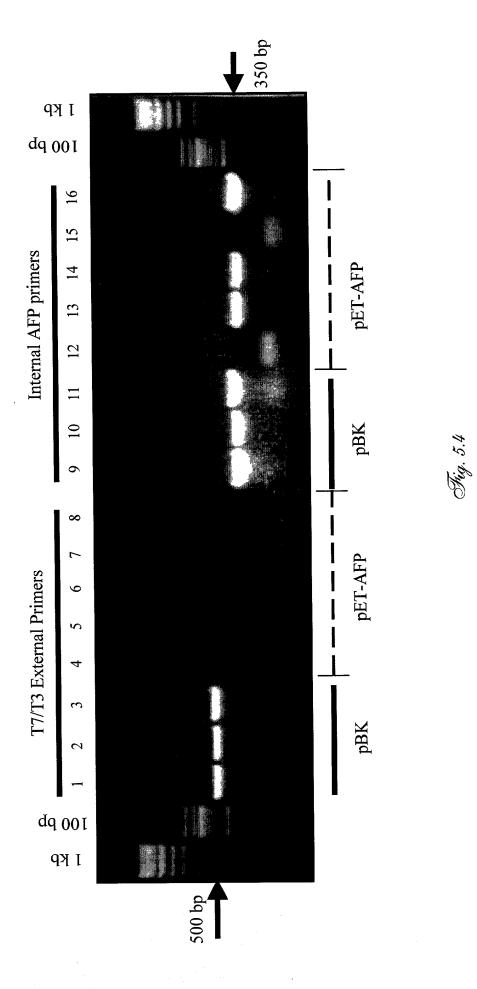


Fig. 5.0









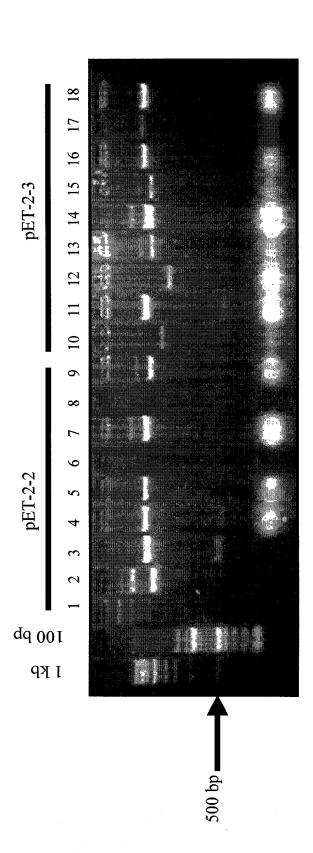


Fig. 5.5

pET-28a Recombinant Histidine cleaved AFP

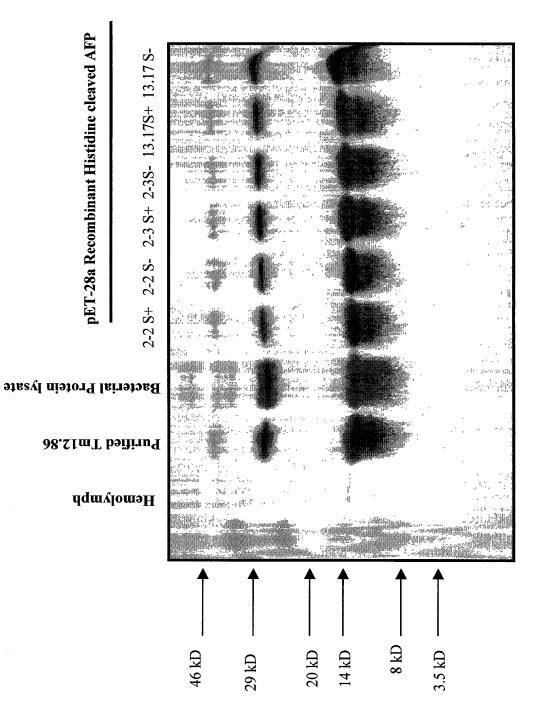


Fig 5.6

His-tagged Clone 2 2 with signal sequence

TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG	50
His-tag Start Codon AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -55 -50	96
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -45 -40 -35	141
AFP Star GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys <u>Met</u> -30 -25 -20	rt Codon 186
AAA CTC CTC TTG TGC TTT GCG TTC GCC GCC ATC GTC ATC GGA GCT Lys Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala -15 -10 -5	231
N-terminal of mature AFP CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser 1 5 10	276
AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp 15 20 25	321
AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys 30 35 40	366
CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala 45 50 55	411
GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val 60 65 70	456
GCC AGC GAC GAA GAG GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val 75 80 85	501
AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys 90 95 100	546
Stop Codon ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTTGTA Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp * 105 110 115	595
Polyadenylation signal Poly-A tail TTTGACTGAA TTTTGAC <u>AAT AAA</u> GGTAATA TCGTTATGTA AAAAAAAAA	645
AAAAAACTCG AGCACCACCA CCACCACCAC TGAGAT	681

His	-tag	ged (clone	e 2.2	2 wit	thou	t sig	gnal	sequ	uence	9				
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				CCG Pro											141
				GGT Gly				CTC	ACC	GAC	GAA		ATA	CAG	186
				ATC Ile											231
				ATC Ile											276
				AAG Lys											321
				GAA G1u											366
				CAT His											411
				GTG Val											456
				AAG Lys											501
		GAT Asp 115	TAA	COC CTC		ACC A	ACCAC	CCACC	CA CO	CACTO	GAGAT	Γ			543

His-tagged clone 2 3 with signal sequence	
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG	50
His-tag Start Codon AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -55 -50	96
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -45 -40 -35	141
AFP Start GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met -30 -25 -20	Codon 186
AAA CTC CTC TTG TGC TTT GCT TTC GCC GCC ATC GTC ATC GGA GCT Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala -15 -10 -5	231
N-terminal of Mature AFP CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC $\underline{\text{Gln Ala}}$ Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser $\underline{\textbf{1}}$ 5 10	276
AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp 15 20 25	321
AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys 30 35 40	366
CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala 45 50 55	411
GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val 60 65 70	456
GCC AGC GAC GAA GAA GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val 75 80 85	501
AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys 90 95 100	546
Stop Codon ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTTGTA Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp * 105 110 115	595
Polyadenylation signal Poly-A tail TTTGACTGAA TTTTGAC <u>AAT AAA</u> GGTACTA TCGTTATGAA AAAAAAAAA	645
AAAAAAACTC GAGCACCACC ACCACCACCA CTGAGAT	682

His-tagged Clone 2.3 without signal sequence	
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG	50
His-tag Start Codon AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -30 -25	96
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -20 -15 -10	141
N-terminal of mature AFP GGA CAG CAA ATG GGT CGC GGA TCC CTC ACC GAC GAA CAG ATA CAG Gly Gln Gln Met Gly Arg Gly Ser Leu Thr Asp Glu Gln Ile Gln -5 1 5	186
AAA AGG AAC AAG ATC AGC AAA GAA TGC CAG CAG GTG TCC GGA GTG Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val 10 15 20	231
TCC CAA GAG ACG ATC GAC AAA GTC CGC ACA GGT GTC TTG GTC GAT Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp 25 30 35	276
GAT CCC AAA ATG AAG AAG CAC GTC CTC TGC TTC TCG AAG AAA ACT Asp Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr 40 50	321
GGA GTG GCA ACC GAA GCC GGA GAC ACC AAT GTG GAG GTA CTC AAA Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys 55 60 65	366
GCC AAG CTG AAG CAT GTG GCC AGC GAC GAA GAA GTG GAC AAG ATC Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile 70 75 80	411
GTG CAG AAG TGC GTG GTC AAG AAG GCC ACA CCA GAG GAA ACG GCT Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala 85 90 95	456
TAT GAC ACC TTC AAG TGT ATT TAC GAC AGT AAA CCT GAT TTC TCT Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser 100 105 110	501
Stop Codon CCT ATT GAT TAA CTCGAGCACC ACCACCACCA CCACTGAGAT Pro Ile Asp * 115	543

His-tagged Tm 13 17 with signal sequence	
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG	50
His-tag Start Codon AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -65 -60 -55	96
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -50 -45 -40	141
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC TGG ATC CAA AGA ATT Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Trp Ile Gln Arg Ile -35 -30 -25	186
AFP Start Codon CGG CAC GAG ACT ACT AAG ATG AAG TTG CTC TGT TGT CTA ATC TCC Arg His Glu Thr Thr Lys Met Lys Leu Leu Cys Cys Leu Ile Ser -20 -15 -10	231
N-terminal of mature AFP CTC ATT CTG TTG GTC ACA GTT CAG GCC CTG ACC GAG GCA CAA ATT Leu Ile Leu Leu Val Thr Val Gln Ala Leu Thr Glu Ala Gln Ile -5 1 5	276
GAG AAA CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly 10 15 20	321
GTG TCG CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG Val Ser Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu 25 30 35	366
GAC GAT CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC Asp Asp Pro Lys Leu Lys Arg G1n Val Phe Cys Val Ala Arg Asn 40 45 50	411
GCC GGT CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG Ala Gly Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu 55 60 65	456
AGG GAG AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG Arg Glu Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu 70 75 80	501
AAA ATC ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG Lys Ile Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu 85 90 95	546
ACG GTG TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG Thr Val Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys 100 105 110	595
Stop Codon TTC TCA CCA GTT GAT TGA ACCACCACGA CTAGTAGATG GTTCAAATGG Phe Ser Pro Val Asp * 115	643
Polyadenylation signal Poly-A tail TGTGCTTTAC ATATAAA <u>AAT AAA</u> GTGTTTC TGATGTAAAA AAAAAAAAAA	693
AAAAAAAAAA AACTCGAGAG TATTCTAGAG CGGCCGCGGG CCCATCGTTT	743
TCCACCCCTC GAGCACCACC ACCACCACCA CTGAGAT	777

His-tagged Tm 13.17 without signal sequence	
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG	50
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -30 -25	96
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -20 -15 -10	141
N-terminal of mature AFP GGA CAG CAA ATG GGT CGC GGC CTG ACC GAG GCA CAA ATT GAG AAA Gly Gln Gln Met Gly Arg Gly Leu Thr Glu Ala Gln Ile Glu Lys -5 1 5	186
CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA GTG TCG Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly Val Ser 10 15 20	231
CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG GAC GAT Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu Asp Asp 25	276
CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC GCC GGT Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn Ala Gly 40 45 50	321
CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG AGG GAG Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu Arg Glu 55 60 65	366
AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG AAA ATC Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu Lys Ile 70 75 80	411
ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG ACG GTG Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu Thr Val 85 90 95	456
TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG TTC TCA Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys Phe Ser 100 105 110	501
Stop Codon CCA GTT GAT TGA CTCGAGCACC ACCACCACCA CCACTGAGAT Pro Val Asp * 115	543

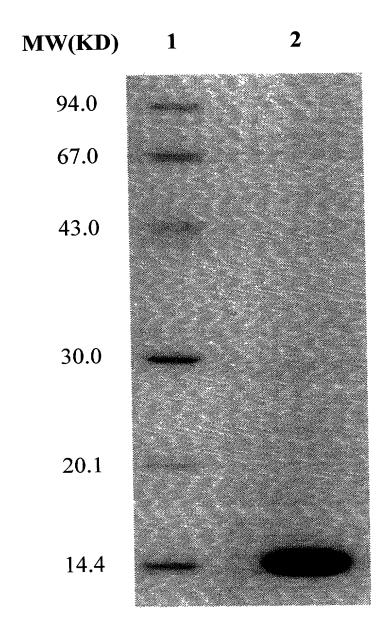


Fig. 6.0

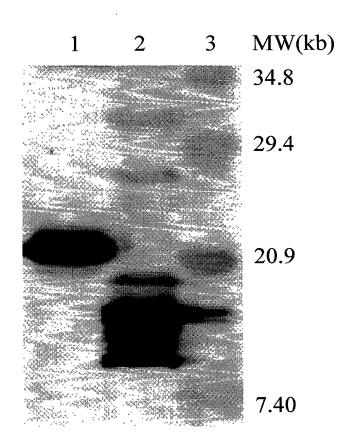


Fig. 6.1

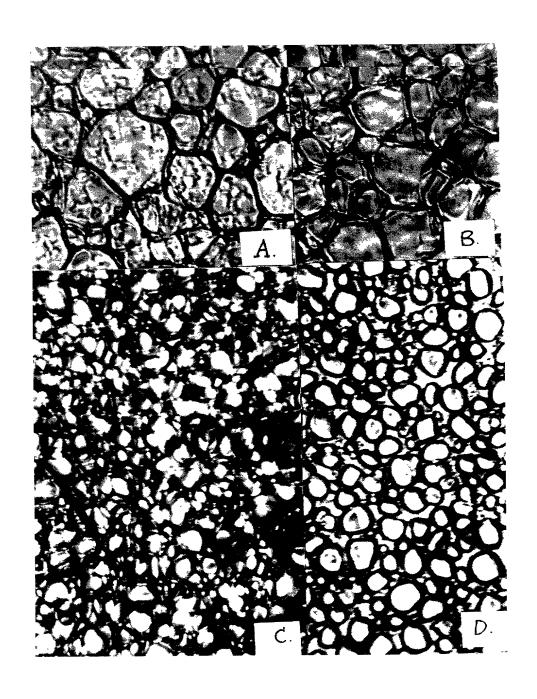
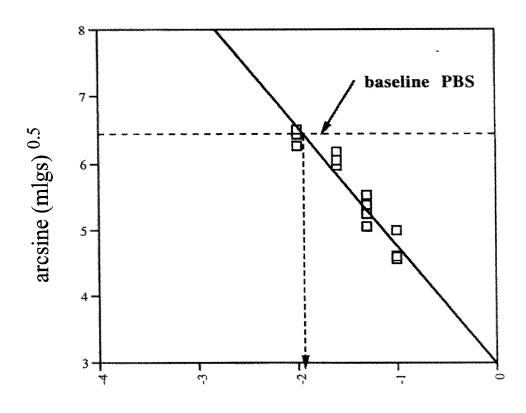


Fig. 6.2

Tm 13.17 S-graph data



log dilution

ONE LETTER	NAME	THREE LETTER	CHEMICAL CLASS	HYDROPHOBICITY	Reactivity
Α	Alanine	Ala	aliphatic	mod. hydrophobic	low
В	Asp or Asn	Asx			
С	Cysteine	Cys	sulfhydryl	hydrophobic	high
D	Aspartic Acid	Asp	acidic	highly hydrophilic	high
E	Glutamic Acid	Glu	acidic	highly hydrophilic	high
F	Phenylalanine	Phe	aromatic	highly hydrophobic	low
G	Glycine	Gly	aliphatic	mod. hydrophobic	low
H	Histidine	His	basic, imidazole	highly hydrophilic	high
ï	Isoleucine	lle	aliphatic	hydrophobic	low
Ĵ			·		
K	Lysine	Lys	basic	highly hydophilic	high
Ĺ	Leucine	Leu	aliphatic	hydrophobic	low
M	Methionine	Met	sulfhydryl	hydrophobic	low
N	Asparagine	Asn	amide, acidic derived	hydrophilic	high
Ö	, iopag		•	• •	
P	Proline	Pro	aliphatic, cyclic, imino	mod. hydrophilic	low
Q.	Glutamine	Gin	amide, acidic derived	hydrophilic	high
R	Arginine	Arg	basic	highly hydrophilic	high
S	Serine	Ser	aliphatic hydroxyl	hydrophilic	high
Ť	Threonine	Thr	aliphatic hydroxyl	hydrophilic	high
Ú	.,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,			• •	
v	Valine	Val	aliphatic	hydrophobic	low
w	Tryptophan	Trp	aromatic	highly hydrophobic	low
X	113610611011				
Y	Tyrosine	Tyr	aromatic	mod. hydrophilic	high
ż	Glu or Gln	Glx		• •	_
_	0.0 0. 0	•			
		ACD	Any Acidic		
		ALP	Any Aliphatic		
		ALH	Any Aliphatic Hydroxyl		
		ARO	Any Aromatic		
		BAS	Any Basic		
		HY-	Hydrophobic		
		HY+	Hydrophilic		

Position	Tm12 84-2 2	Tm12 84-2 3	Tm12 84-3 4	Tm12 84-3 9	Tm12 84-7 5	Concensus of Tm12 84	Tm13 17	Concensus with Tm 13 17	В1	Concensus with B1	AFP-3	Concensus with AFP-3
2 3	A C G	A C G	A C G	A C G	A C G	A C G	A G · A	A N R		A N R	C A G	N N R
5	A G	A G	A G	A G	A G	A G	C T	N N		N N	A T	N N
- 6 7 8	C A A	C A	C A	C A	C A	C A	Ĉ.	N N N		N N	CCC	N N N
9	Ä	A A A	A A A	A A A	A A A	A A	A A	A A		N A A	G A A	A A
11 12	Ã	Ã	Ā	Ā	Ā	Ã	Ğ	R •		Ř	Ĝ	Ř •
13 14	A T	A T	A T	A T	A T	A T	A T	A T	A? T?	A T	A T	A T
15 16	G A	G A	G A	G A	G A	G A	G A	G A	G?	G A	G A	G A
17 18 19	A A C	A	A A C	A A C	A A C	A A C	A G	A R Y		A R Y	A G	A R Y
20	Ť	C T C	T C	T C	Ť	T C	T T G	C/G		T C/G	C T C	T C/G
22 23	C T	C T	C T	C T	C T	C T	C T	C T		C T	Ç	C T
24 25	C T	C T	C T	C T	C T	C T	C T	C T		C T	C C	C Y
26 27	r G	Ğ.	T G	T G	Ğ G	T G	G T	N N		N N	Č	N N
28 29 30	T G C	T G C	T G C	T G C	T G C	G G	G T	G Y		T G Y	G T	T G Y
31 32	Ť	T T	T T	T T	T T	Ť	Ċ	Ý T	C T	Ý T	ċ	Ý T
33 34	T G	T G	T G	T G	T G	T G	Á	T/A R	T A	T/A R	T G	T/A R
35 36	c G	C T	C I	C T	ç G	C N	T C	Ň	C T	Ň	Č.	Y N
37 38 39	T T C	T T C	T T C	T T	T C	т т с	C C	Y C	T C T	Ť Y Y	C T C	Y
40 41	Ğ	G	G C	C G C	G	G	Ċ	άς	Ċ	G/C Y	G T	G/C Y
42 43	C G	C G	C G	C G	Ċ	C G	Ċ A	C R	Ċ A	Ċ R	Ť G	Y R
44 45	c	c c	c	c c	c	C	T T	Y Y	T T	Y	c	Y
46 47 48	A T C	A T C	A T C	A T C	A T C	A T C	C T G	N T C/G	C T G	N T C/G	Ť T G	N T C/G
49 50	Ğ	Ğ	Ğ T	Ğ T	Ğ	Ğ	Ť	N T	Ť	N T	G T	N T
51 52	C A	C A	C A	C A	C A	C A	G G	C/G R	G G	C/G R	C G	C/G R
53 54 55	T C	T C	T C	T C	T C	T C	Č.	T C	T C	T C	c	Y C
56 57	G G A	G G A	G G A	G G A	G G A	G G A	A C A	R G/C A	C C A	N G/C A	G C G	N G/C R
58 59	G C	G C	Ğ	Ğ	Ğ	G C	Ĝ	Ğ Y	Ğ	Ĝ	A C	Ŕ
60 61	T C	T C	T C	T C	T C	T C	T C	T C	T C	T C	C T	Y
62 63 64	A G G	A G G	A G G	A G G	A G G	A G G	A G G	A G G	A G G	A G G	A C G	A N G
65 66	C T	C T	C T	Ċ	C T	c T	c	C Y	c	C Y	000	Ċ
67 68	C T	C T	C T	C T	, C T	C T	C T	C T	A T	N T		N T
69 70 71	C A C	C A C	C A C	C A C	C A C	C A C	G A	C/G A C	A A	N A		N A
72 73	Ċ	C	C	C	Ċ	C	C G	C G	C T C	ekc A C	G	C Y G/C
74 75	Ā C	A C	Ã	Ā	Ā C	A Y	A G	A N	A G	A N	Ä	A N
76 77	G A	G A	G A	G A	G A	G A	G C	G N	G A	G N	A C	R N
78 79 80	A C A	A C A	A C A	A C A	A C A	A C A	A C A	A C A	A G A	c <i>i</i> G	A C	C/G
81 82	Ĝ	Ĝ	Ĝ	Ĝ	Ĝ	Ğ	Ä	R A	Č	A N N	C T C	N N N
83 84	T A	T A	T A	T A	T A	Ť A	T T	T A/T	Ť	T A/T	G T	N A/T
85 86 87	C A G	C A G	C A G	C A G	C A G	C A G	G A	C/G A G	G A G	C/G A G	G A	C/G A
88		A A					A	A A	C T	N A/T	G A	N Arr
89 90 91	A A	A A G G	A A	A A	A A	A A	A A A C T G A A C A A G A T C	A N	Ä	A N N	Ĝ	R N
92 93 94 95 96 97 98 99	G G	G G	G G	G G	G G	G	T G	N G	T G	N G	T G	N G
95 96	A A	A A	Ä	A	A A	A A	Ä	A A C	C	20	Â	N N
97 98	A A	A A	Ā	Ä	Ä	A A	Ä	A A G	Č A	N A	G A	N A
99 100	G A	G A	G A	G A	G A	G A	G A	G A	A A	R A	G C	R N
101 102 103	A A A A G G A A C A A G A T C A G C A A A G A A T	C A	C A	T C A	T C A	C A	C A	A T C	C	C A	A C	N C
102 103 104 105	G C	Ĝ	Ĝ	GC	Ĝ	Ĝ	A G C A A A	A G C	Ğ	G C	Ğ	G C
106 107	A	A A	A A	A A	A A	A A	A	A A A R	G	R N	G A	Ř N
108 109 110	A G A	A G	A G	A G	A G	A G	A	A R	A G	A R	G	N R
111 112	Ä	Ã	A T	Ã	Ğ	Ř Ť	A T	A R T	Ğ	R T	C T	N N T
113 114	G C	G C	G C	G C	G C	G C	A T G T C A	G Y	Ġ	G Y	G C	A R N N G N N Y N A R N N O A G O R N N R N N T G Y N
115 116 117	G C C A G	A A C A A G A A T C C C A G	A A A A G G A A C A A G A T C A G C A A A G A A T G C C A G	A A A A G G A A C A A G A T C A G C A A A G A A T G C C A G	A A A A G G A A C A A G A T C A G C A A A G A G T G C C A G	A A A A G G A A C A A G A T C A G C A A A G A R T G C C A G	C A A	G Y C A R	A C T G C C C C A A A C C A G C G C A G A G T G C A A G	0 N N C N A R A Y C A G C R N A R A R T G Y N A R	A A G C T G A A G C A G C A G C G A C G C C T G C A A G	N A R

											AFP-3	Concensus with
Position	Tm12 84-2 2	Tm12 84-2 3	Tm12 84-3 4 C	Tm12 84-3 9 C	Tm12 84-7 5 C	Concensus of C	Tm13 17 A	Concensus with N	B1 A	Concensus with N	G	N
118 119	C A	C A	A G	A	Α	Α	A T	A N	C T	N N	C T	N N
120 121	G G	G G	G G	G G	G G	G G	G	G	G	G	G	G
122	Т	T	T	Α	T G	T/A G	A A	T/A R	A A	T/A R	A G	A/T R
123 124	G T	G T	G T	G T	T	T	Α	T/A	Α	T/A	T	T/A
125 126	C C	C	c	c	C	c	G T	C/G Y	G T	C/G Y	C T	C/G Y
127	G	G	G	G	G	G	G	G	G G	G G	G G	G G
128 129	G A	G A	G A	G A	G A	G A	G A	G A	Α	A	Α	A
130	G	G	G	G	G T	G T	G T	G G	G T	G T	G T	G T
131 132	T G	T G	T G	T G	Ġ	G	G	G	G	G	A T	R T
133	T	T C	T C	T C	T C	T C	T C	T C	T C	T C	С	С
134 135	c	C	С	C	С	C	G C	C/G	C G	C/G C/G	T G	N Ç <i>I</i> G
136 137	C A	C A	C A	C A	C A	Ä	Ä	Α	Α	Α	A	Α
138	Α	A	Α	A G	A G	A G	A G	A G	A G	A G	A G	A G
139 140	G A	G A	G A	Α	Α	A	Α	Α	A C	A G/C	A G	A G/C
141 142	G A	G A	G A	G A	G A	G A	G A	G A	G	R	T	N Y
143	С	С	С	С	C	С	T C	G/C Y	T C	G\C Y	C C	G/C Y
144 145	G A	G A	G A	G A	G A	G A	Α	A	A	Α	С	N T
146	Т	T C	T C	T C	T C	č	T A	T N	T A	T N	T C	N
147 148	C G	G	Ğ	G	G	G	Α	R	A	R N	A	R N
149 150	A C	A C	A C	A C	A C	A C	c	N C	A G	C/G	С	C/G
151	A	Ā	À	A	A	A	A A	A A	A G	A R	A A	A R
152 153	A A	A	A A	A A	A A	A	A	A	Α	A	G	R
154 155	G T	G T	G T	G T	G T	G T	G C	G Y	G C	G Y	G T	G Y
156	С	С	С	С	c	С	Т	Y C	T C	C Y	T C	Y C
157 158	C G	C G	C	C G	C G	C G	C G	G	G	G	G	G
159	C	C	С	C A	C A	C A	C A	C A	C A	C A	C A	C A
160 161	A C	A C	A C	ĉ	С	С	Α	N	A	N N	A C	N N
162 163	A G	A G	A G	A G	A G	A G	C G	N G	A G	G	Ċ	G/C
164	G	G	Ğ	Ģ	G T	G	G T	G T	G T	G T	G T	G T
165 166	T G	T G	T G	T G	Ġ	Ġ	G	G	G	G	Ğ	G T/A
167	T	T C	T C	T C	T C	T C	A C	T/A C	A C	T/A C	A A	N
168 169	C T	Т	T	T	Ţ	Т	т	T	т т	T N	G A	N N
170 171	T G	T G	T G	Ť G	T G	e T	G G	N G	G	G	A	R
172	G	G	G	G T	G T	G T	G A	G T/A	G A	G T/A	G T	G T/A
173 174	T C	T C	T C	Ċ	Ċ	С	G	C/G	G	C/G	G	C/G
175 176	G	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A
177	A T	С	С	C	С	Y	С	Y G	C G	Y G	C G	Y G
178 179	G A	G A	G A	G A	G A	G A	G A	Α	A	Α	Α	A
180	Т	T C	T C	T C	T C	T C	T C	Ť C	c	Y C	C	č
181 182	C	С	C	c	С	Č	Ċ	С	c	C Y	C	C Y
183 184	C A	C A	C A	C A	C A	C A	T A	Y A	C A	Ä	Α	A
185	Α	A	Α	Α	A A	A	A A	A A	A A	A A	A A	A A
186 187	A A	A A	A A	A A	A	Α	С	N	Ç T	N T	C T	N T
188 189	T G	T G	T G	T G	T G	T G	T G	T G	Ġ	G	G	G
190	A	Ā	A A	A A	A A	A	A A	A A	A	A A	A	A A
191 192	A G	G	G	G	G	G	A	R	A	R N	A G	R N
193 194	A	A A	A A	Â	A	A	C G	N R	T	N	Α	N
195	G	G	G C	G C	G C	G C	c	G/C C	G C	G/C C	G C	G/C C
196 197	C A	C A	A	A	Α	A	Α	A N	A A	A N	A T	A N
198 199	c G	C G	C G	C G	C G	C G	A G	G	ĉ	G/C	Ġ	G/C
200	т	Ŧ	Т	T C	T C	T C	T T	T Y	T T	T Y	C T	Y
201 202	c c	c c	c	С	С	С	T	Y	ċ	Y T	Ţ	Y T
203 204	T C	T C	T C	C	T C	T C	T T	T Y	Ť	Ý	ċ	Y
205	Т	Ť	T	т	T	Т	T	T G	T G	T G	T G	T G
206 207	G C T T	G C	C	C	c	c	č	G C	С	С	G C A T C T	G C X + Z Z Y
207 208 209	T T	T	Ŧ	T T	T T	T T	G T	N T	A T T	N T	T	T T
210	ċ	ċ	ċ	ç	ç	ç	G	C/G	T	N N	C T	N N
210 211 212	С Т С	Č	Ċ	ċ	ć	ċ	c	N C G/C	Т Т С	Y	T	Y
213	G	G	G	G A	G A	G A	C	G/C A	C A	G/C A	G A	G/C A
214 215	Â	Â	Â	Â	Ã	Ä	Ğ	R	A	A R G R	A	R
216 217 218	G A	G C T T C T C G A A G A A A C T G G	G A	G A	G A	G A	G A	Ā	G	R	G A A G A G C C G G A T	Ř
218	Ä	Ā	A	G	A	R	A C	R N	C A	N	G A	N N
219 220	A	Ä	Ã	Ä	Â	Ã	Ğ	R	č	N	G	Ņ
221	C	C T	C T	C T	C T	C T	c	C Y	C	Y	Č	Ϋ́
223	Ġ	Ġ	Ġ	Ġ	Ğ	Ġ	G	G	G A	G R	G	G R
224 225	G A	G A	G A	G A	G A	A	T	ĀT	Ä	A/T	Ă	ĄŢ
226	G	G	G	G T	G T	G T	C T	G/C T	A T	N T R	T	N T
228	G	Ġ	Ġ	Ġ	Ġ	Ġ	Ġ	Ğ	Á	R	С	N
229 230	G C	G C	G C	G C	G C	G C	C	c	T	G Y N	A T	9
220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235	G A A G A A A A C T G G A G T G G C A A C C G	A G T G G C A A C C G	G C T T C T C G A A G A A A A C T G G A G T G G C A A C C G	G C T T C T C G A A G A G A A C T G G A G T G G C A A C C G	G C T T C T C G A A G A A A C T G G A G T G G C A A C C G	G C T T C T C G A A G A R A A C T G G A G T G G C A A C C G	G C G T G G C C A G G A A C G C C G G T C T G G C C A C G G	ARGARNRCYGGATICTGGCNAC	A A G G C A C T C G A A A T A G T C G C G G	N R	C G	GC ARGRNNNYYGR AN TNRYNRNGG G
232	Ĉ	ĉ	ĉ	ĉ	Ĉ	ĉ	ć	C C	č	R C	G A C	N C/G
234 235	C G	C	C G	G	C G	G	G	C/G G	G	C/G G	G	G

Position	Tm12 84-2 2	Tm12 84-2 3	Tm12 84-3 4	Tm12 84-3 9	Tm12 84-7 5	Concensus of	Tm13 17	Concensus with	B1	Concensus with	AFP-3	Concensus with
236 237	A A	A A	A A	A A	A A	A A	A	A	A	A A	C	N N
238 239	G C	0 0 0	о С	G C	G C	G	T C	N C	T C	N C	A G	N C/G
240 241	C G	G	G	G	c G	c G	G G	C/G G	G	C/G G G	T G	N G G
242 243	G A	G A	G A	G A	G A	G A	G A	G A G	G A G	A G	G T G	A/T G
244 245	G A	G A	G A C	G A C	G A C	G A C	G A G	A C/G	A A	A N	A A	A N
246 247 248	C A C	C A C	A C	Ā	A C	A C	G	R Y	Ã	R Y	Ť	N Y
249 250	Č A	C A	Č A	Č A	Č A	Č A	G G	C/G R	T G	N R	Ċ	N N
251 252	Ā	A T	A T	A T	A T	A T	T G	A/T N	A G	A/T N	A A	A/T N
253 254	Ġ	G T	G T	G T	G T	G T	G	G T	G	G Y	C T	G/C Y
255 256	G G	G	G G	G G	G G	G	C G	G/C G	C G	G/C G	G G	G/C G
257 258	A G	A G	A G	A G	A G	A G	A C	G/C	A C	A G/C	A C	A G/C
259 260	G T	G T	G T	G T	G T	G T	G T	G T	A C	R Y	C A	N N N
261 262	A C	A C	A C	A C	A C	A C	G T	R Y T	G T T	R Y T	C A T	N T
263 264	Č.	T C A	T C	T C A	T C A	T C A	T G A	C/G A	Ċ A	cig A	† A	N A
265 266 267	A A A	Â	A A A	Â	Â	Â	Ĝ	Ř R	Ä	R R	A G	R R
268 269	Ĝ	Ĝ	Ĝ	G	Ĝ	Ĝ	Ğ	G N	Ğ	G N	Ä	R N
270 271	Č A	Č A	C A	C C A	C A	C A	G A	C/G A	G A	C/G A	G A	C/G A
272 273	A G	A G	A G	A G	A G	A G	A G	A G	A G	A G	A A	A R
274 275	C T	C T	C T	C T	C T	C T	G T	C/G T	Ţ	N T	Ť	N T
276 277	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A	C A	G/C A N
278 279	A G	A G	A G	A G	A G	A G	G G	R G	C G	N G	A G	N G N
280 281	C A	C A	C A	C A	C A T	C A T	A A	N A N	A G G	N R N	G A A	R N
282 283	T G	T G	T G	T G	Ġ	Ğ	G G T	Ğ	Ť G	N N	Ä	N N
284 285 286	T G G	T G G	T G G	T G G	G	Ġ	Ċ A	G/C R	Å	N N	C T	N N
287 288	c	Ċ	c	o c	c	Ċ	C T	Ç	Ä	N N	Ċ	N N
289 290	Ä G	Ă G	A G	Ä G	A G	Ä G	Ġ A	R R	A	R N	G A	R N
291 292	č	č	Č	č	Ċ	Ċ	C A	C A	G A	C/G A	G G	C/G R
293 294							A C	A C	T G	A/T C/G	A T	A/T N
295 296	G A	G A	G A	G A	G A	G A	G A	G A	A T	R A/T	Č.	N N
297 298	c G	C G	C G	C G A	C G A	C G A	C G A	C G A	G A A	C/G R A	G G A	C/G R
299 300 301	A A G	A A G	A A G	A G	A G	Â G	Â	Â	Ĝ	Ř R	Ĝ	A R R
302 303	Ä G	Ä	Ä G	Ā	Ā	Ä R	Ā	A R	A	A R	A G	A R
304 305	G T	G T	G T	G T	G T	G T	A C	R Y	G C	R Y	G T	R Y
306 307	G G	G G	G G	G G	G G	G G	T G	N G	G A	N R	C G	N R
308 309	A C	A C	A C	A C	A C	A C	A G	c/G	G A	R N	A T	R N
310 311	A A G	A A G	A A G	A A G	A A G	A A G	A A A	A A R	A G A	A R R	G A C	R R N
312 313 314	A T	A T	A T	A T	A T	A T	Ā	Ä	Î	A/T T	Ť	A/T T
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317 318	T G	T G	T G	T G	T G	T G	T C	R T G/C	C G	G/C Y	T T	Y N
319 320	C A	C A	C A	C A	C A	C A	A	N A	A G	N R	G C	N N
321 322 323	G A	G A	G A	G A	G A	G A	T A	N A	A A G	N A	C A	N A B
323 324	G T	G T	G T	G T	G T	G T	Ğ	G		N N	A T	N N
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330 331	G G	G G	G G	G G	G G	G G	C G	G/C G	G T	G/C N	C G T	G/C N N N N D G
332 333	T C	T C	T C	C T	T C	T C	T C	T C	G A	N N	С	N N
334 335	A	A A	A	A	A	A A	A	A A	C T	N N	A A G	N N
336 337	G A	G A	G A	G A	G A	G A	G A	G A	A A	G A	A A	A B
324 325 326 327 328 339 331 332 333 334 335 336 337 338 341 342 343 344 345 343 344 345	G G	G G	G G	A G	G	G	A	R G	C A	A R N R	A G G	A R N R N N N
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347 348	C A	C A	C A	C A	C A	C A	T T	A/T	G G	C/G N N R A R R	C T	N N
349 350 351	G A	G A	G A	G A	G A	G A	G A	G A	Ā	R A	C A	N A
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Position	Tm12 84-2 2	Tm12 84-2 3	Tm12 84-3 4	Tm12 84-3 9	Tm12 84-7 5	Concensus of	Tm13 17	Concensus with	81	Concensus with	AFP-3	Concensus with
354 355	A A	Α	A A	A A	A	A	G A	R A	A C	R N	C T	N N
356	c G	A C G	A C G	A C G	A C G	A C G	C G	C G	G C	C/G G/C	C	C/G G/C
357 358	G	G	G	G	G	G	G T	G Y	C A	G/C N	T C	N N
359 360	C T	C T	C T	C T	C T	C T	G	N	T	N	A	N
361 362	T A	T A	T A	Т А	T A	T A	T T	T A/T	T T	T A/T	G C	N N
363	T	T	T	T	7	T	С	Y	G A	N R	C G	N R
364 365	G A	G A	G A	G A	G A	G A	A A	R A	Α	A	A	Α
366	С	C A	C A	C A	C A	C A	T A	Y A	G T	N A/T	C T	N A/T
367 368	A C	С	С	C	С	C	C T	C Y	T A	Y N	T C	Y N
369 370	C T	C T	C T	C T	C T	T	Т	T	C	Y	T	Ÿ Y
371 372	T C	t C	C A	Y N	T T	N						
373	Α	Α	Α	A	Ä	A A	A A	A	A	A A	A A	A A
374 375	A G	A G	A G	G	G	G	A	R	T G	N N	A T	N N
376 377	T G	T G	G T	T G	T G	N N	T G	N N	T	N	G	N
378 379	T	T	T A	T A	T A	T A	T G	T R	G T	N N	C G	N N
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383 384	A C	A C	A C	A C	A C	A C	T G	A/T C/G	G A	N N	A C	N N
385	G	G	G	G	G	G	Α	R A	A G	R R	G A	R R
386 387	A C	A C	A C	A C	A C	A C	A	N	G	N	T	N
388 389	A G	A G	A G	A G	A G	A G	A A	A R	A C	A N	A A	A N
390	С	т	Т	Ŧ	т	T	C A	Y A	A A	N A	C A	N A
391 392	A A	A A	A A	A A	A A	A	A	A	G	R	G	R
393 394	A C	A C	A C	A C	A C	A C	G C	R C	c	N C	G T	N Y
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403 404	C T	C T	T T	Ϋ́Τ								
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420 421	T T	T T	T T	T T	T T	T T	A C	T/A Y	C T	N Y	T G	N N
422	G	G	G	G	G T	G T	C A	G/C T/A	G A	G/C T/A	G G	N N
423 424	T T	T T	T T	Ţ	T	T	C	Y	Α	N N	Ğ	N N
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437 438	G A	G A	G A	G A	G A	A	T	Α·T	Ä	A/T	Т	A/T
439 440	A T	A T	A T	A T	A T	A T	G G	R N		R N	A T	R N
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Position	Tm12 84-2 2	Tm12 84-2 3	Tm12 84-3 4	Tm12 84-3 9	Tm12 84-7 5	Concensus of	Tm13 17	Concensus with	B1	Concensus with	AFP-3	Concensus with
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473	A	A	A	A	Α	A	A	A		A	Α	A
474	Ť	T	T	T	Т	T	T	т		Т	Т	7
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476	2	Ä	Ä	Â	Ä	A	A	A		A	Α	A
477	Ä	Ä	A	A	A	A	A	A		A	Α	Α
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Fig. 7.3 cont.

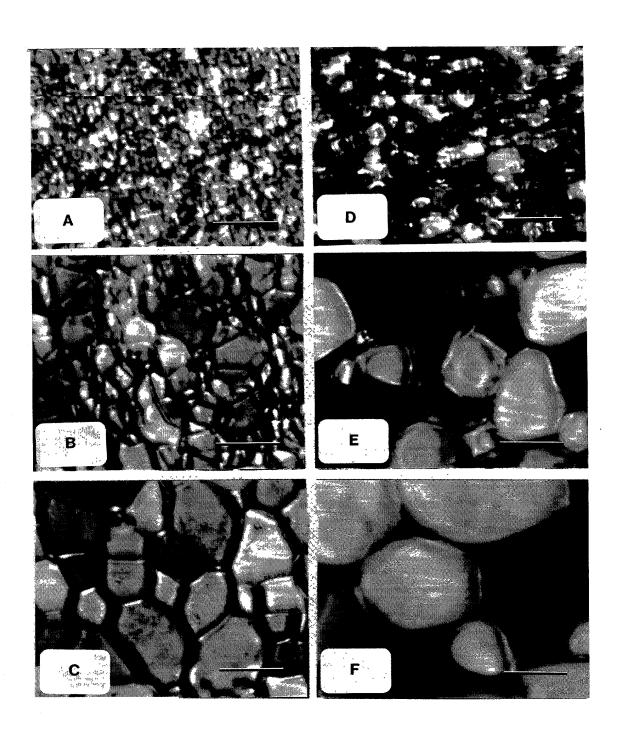


Fig. 8.0

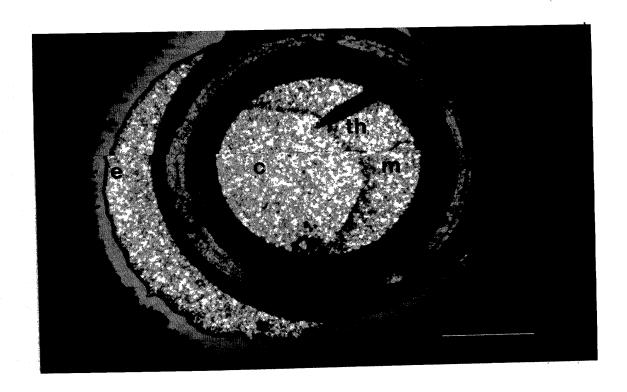


Fig. 8.1a

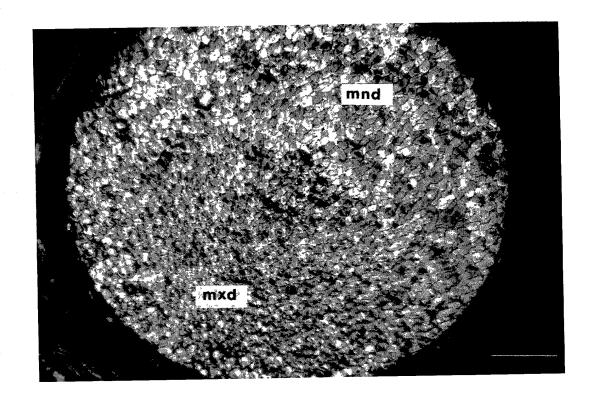
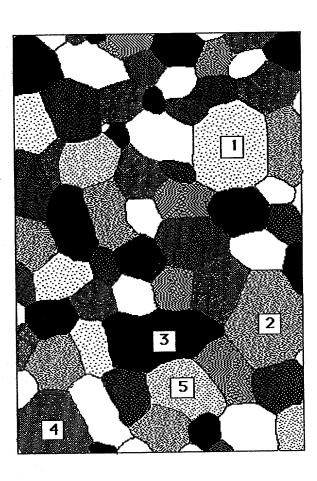
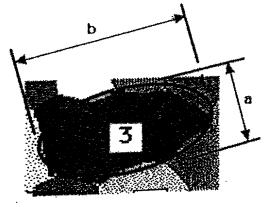
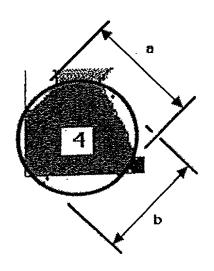


Fig. 8.1b





grain area=0.25mab



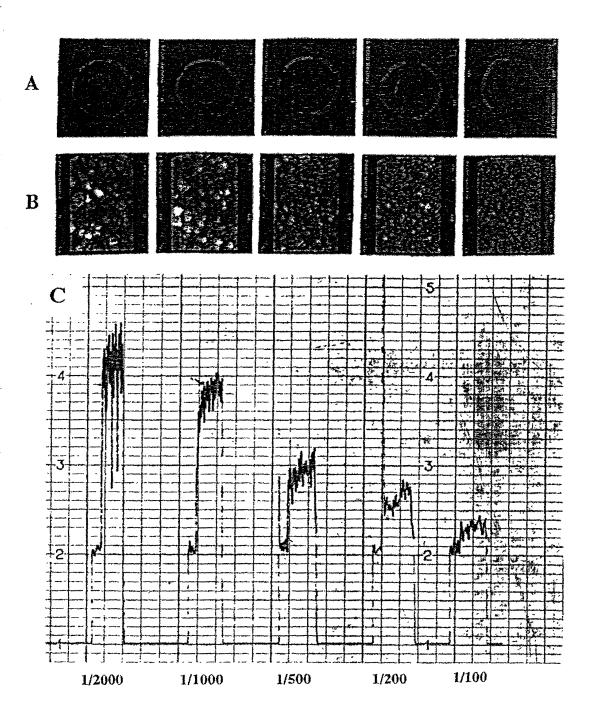
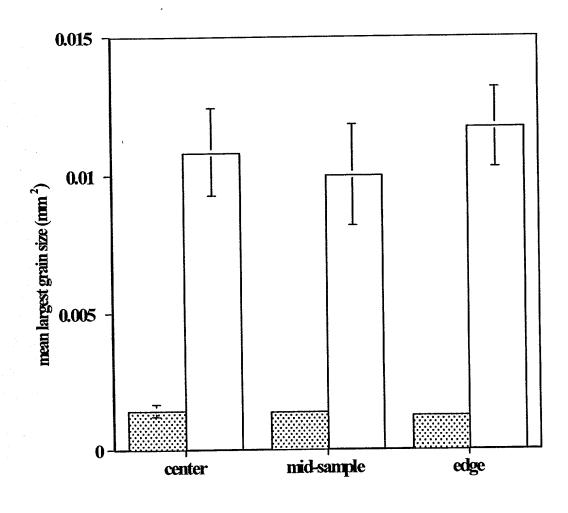


Fig. 8.3



category

Fig. 8.4a

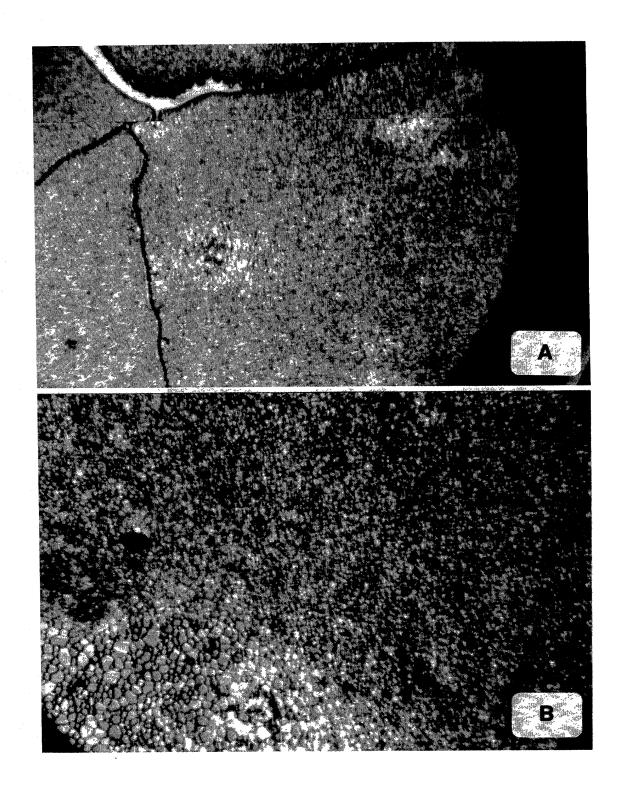


Fig. 8.4b

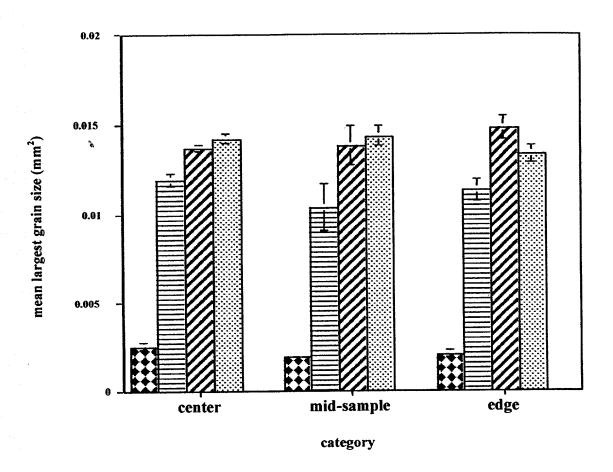


Fig. 8.5a

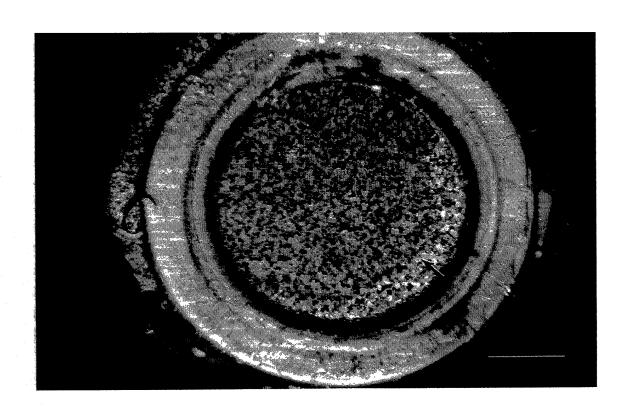


Fig. 8.5b

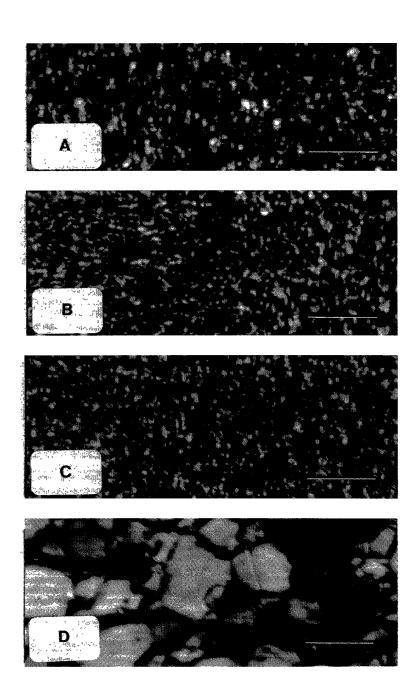


Fig. 8.6

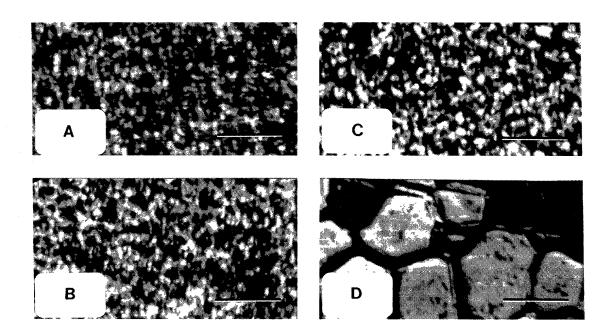


Fig. 8.7

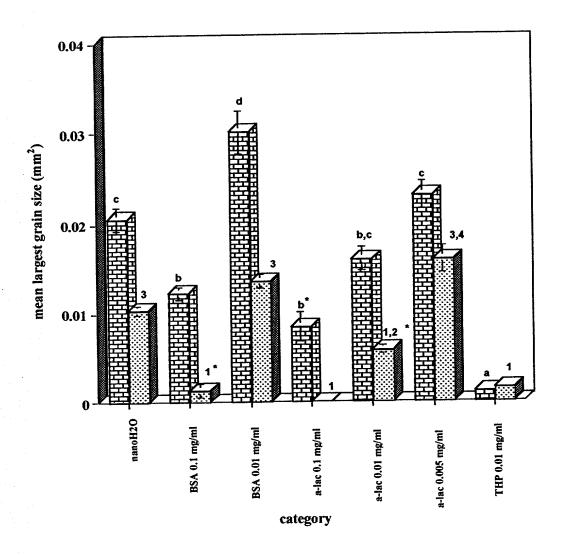


Fig. 8.8

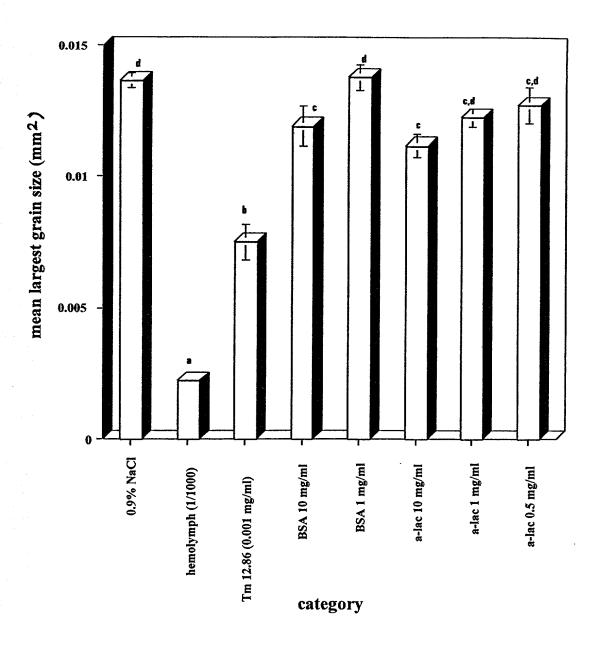
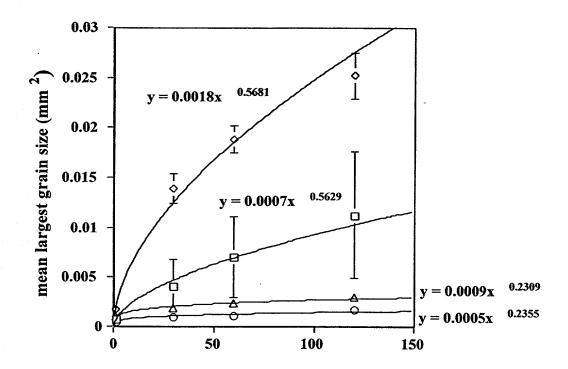


Fig. 8.9



time (minutes)

Fig. 8.10

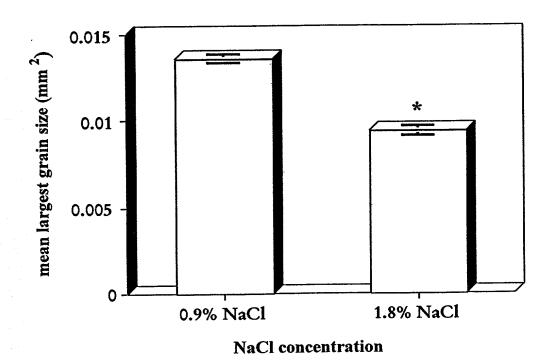


Fig. 8.11

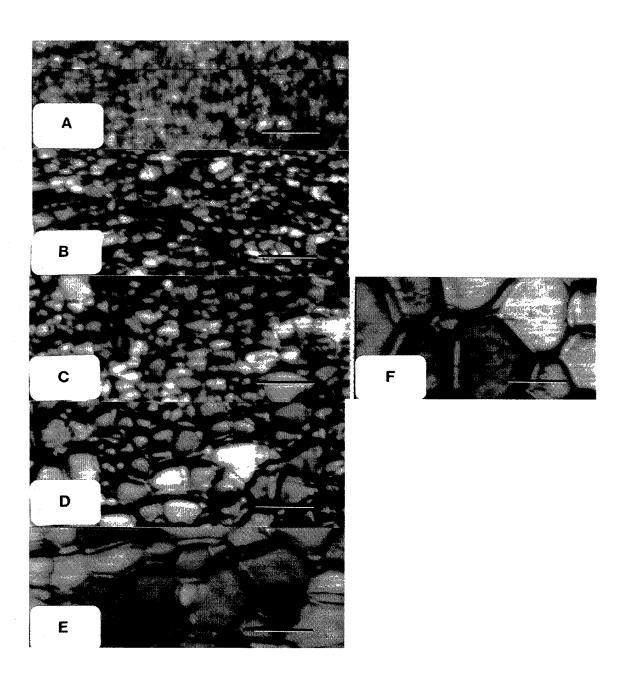


Fig. 8.12

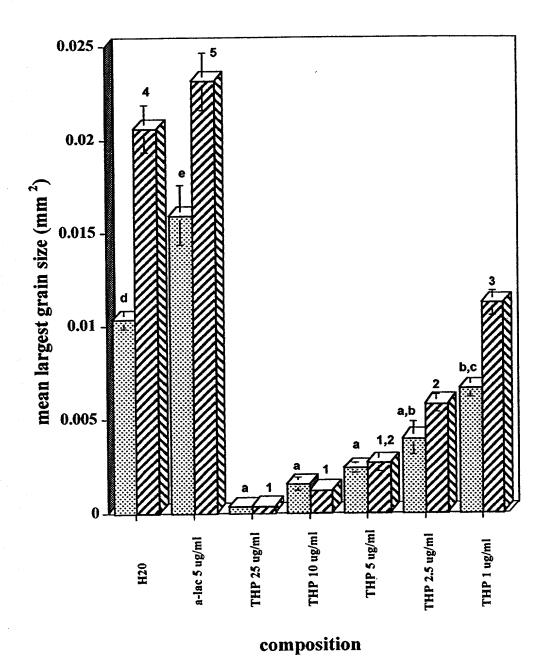


Fig. 8.13

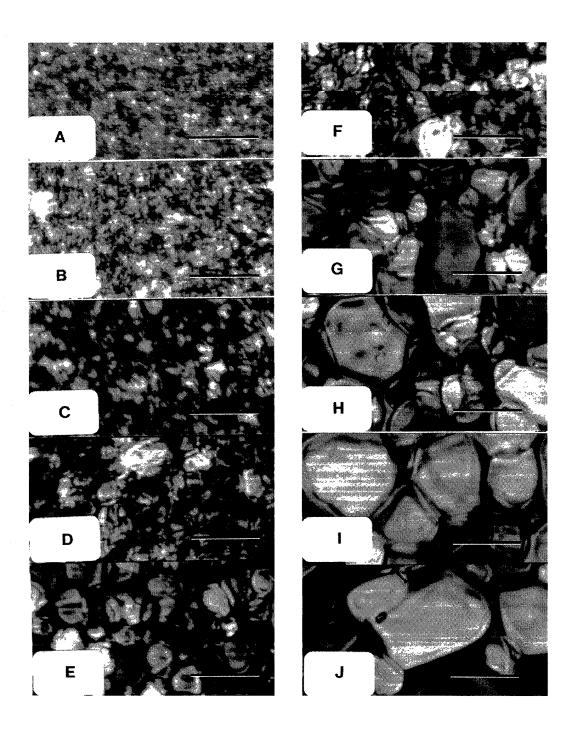


Fig. 8.14

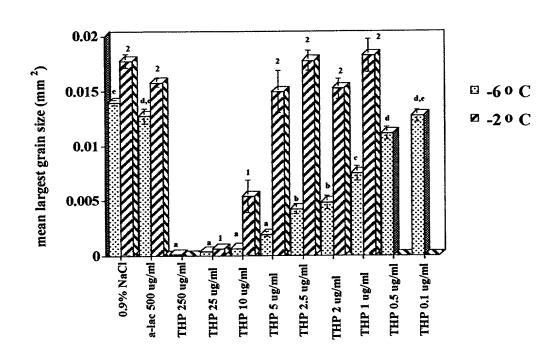
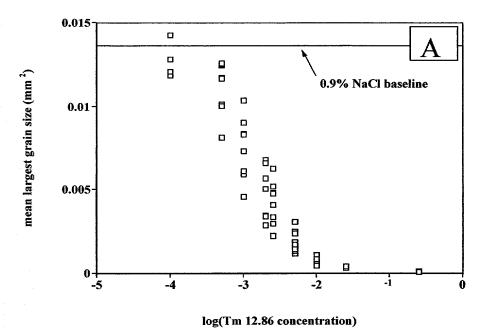


Fig. 8.15



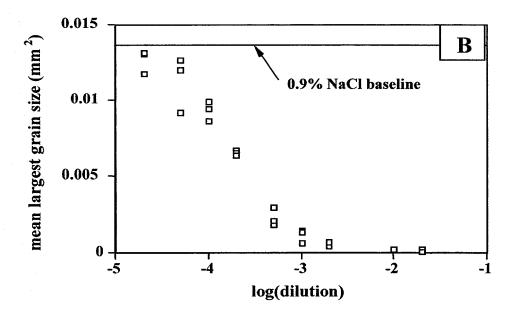
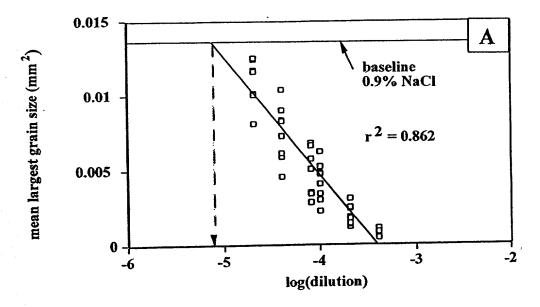


Fig. 8.16



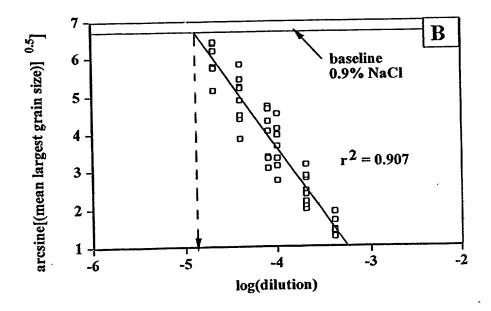
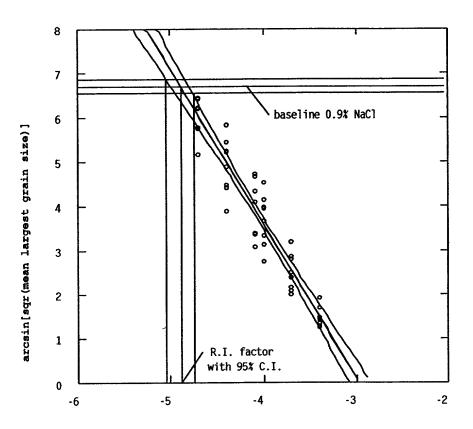
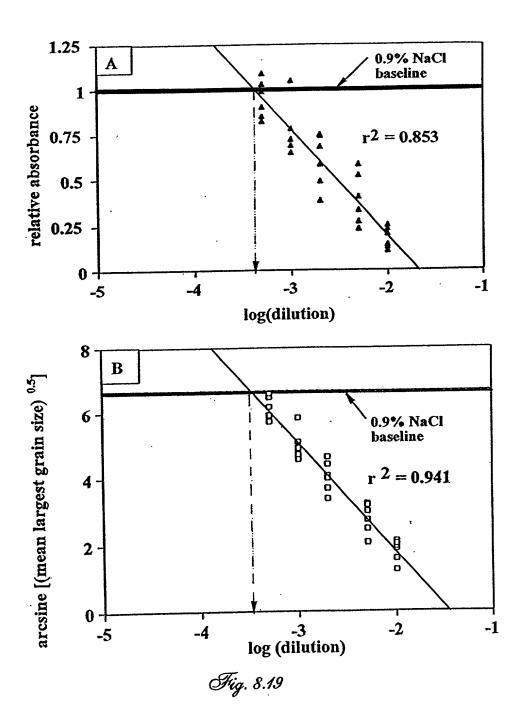


Fig. 8.17



log(dilution)

Fig. 8.18



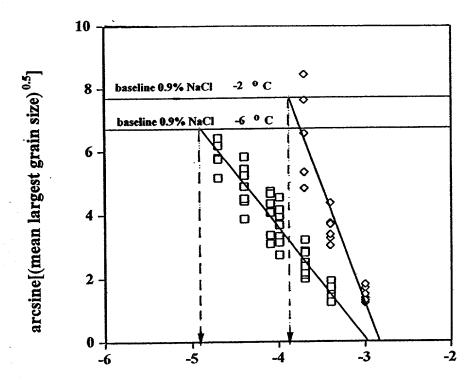


Fig. 8.20

log(dilution)

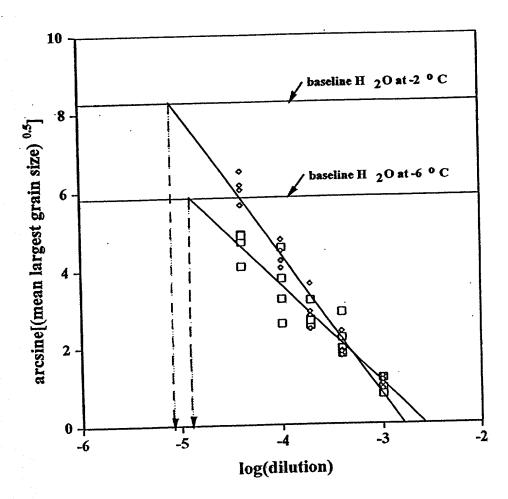
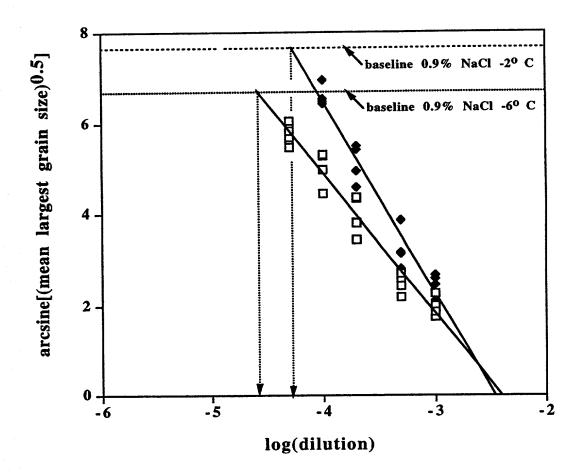


Fig. 8.21



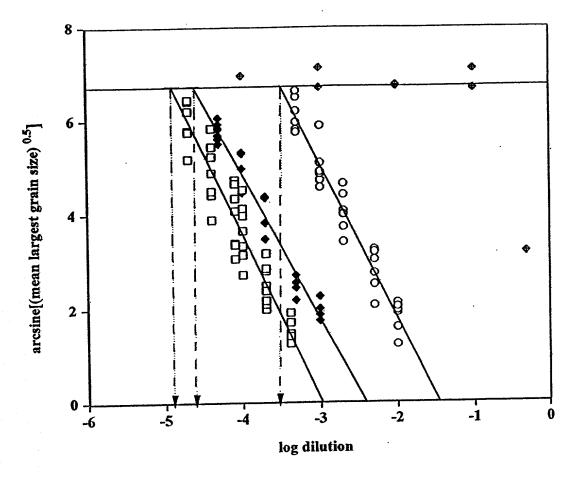
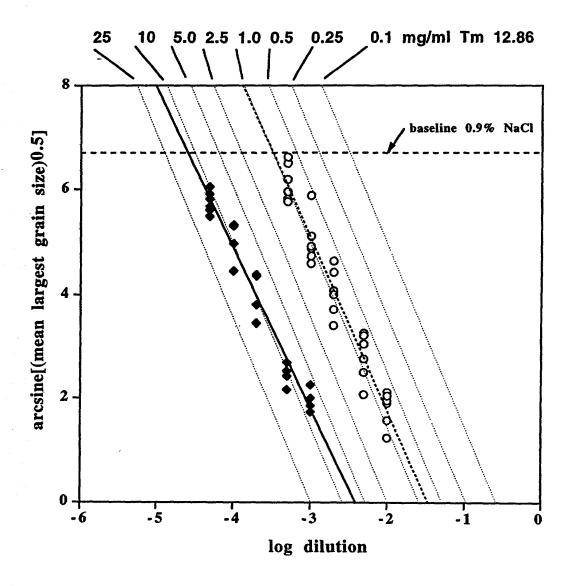


Fig. 8.23



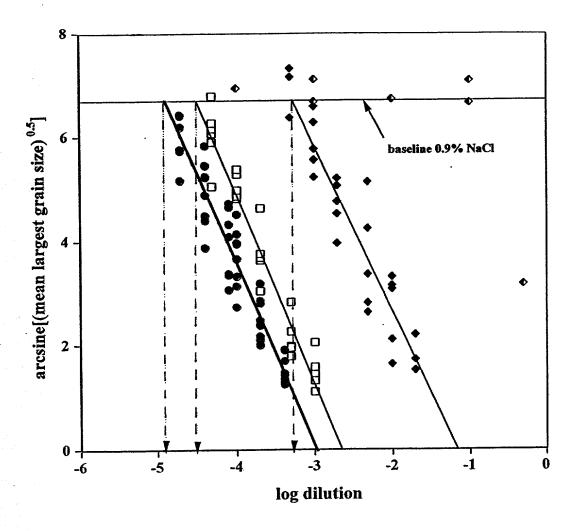


Fig. 8.25

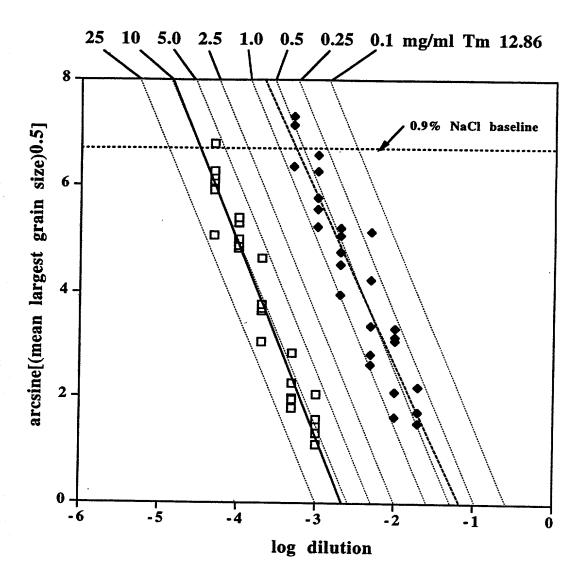


Fig. 8.26

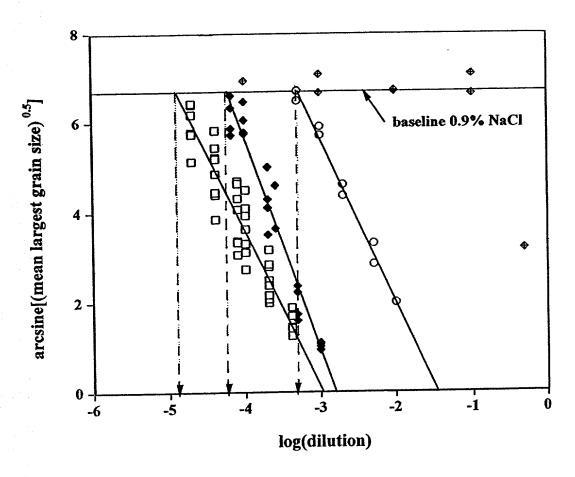


Fig. 8.27

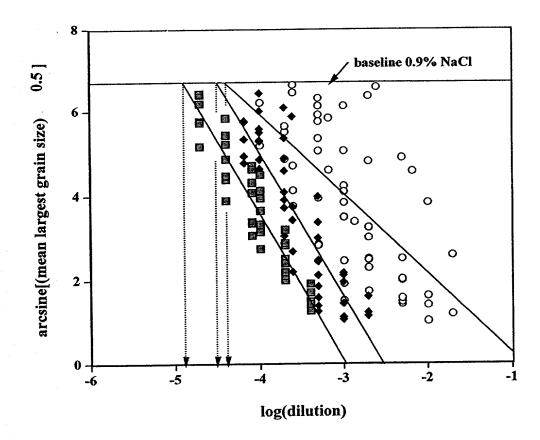
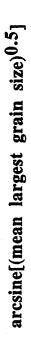
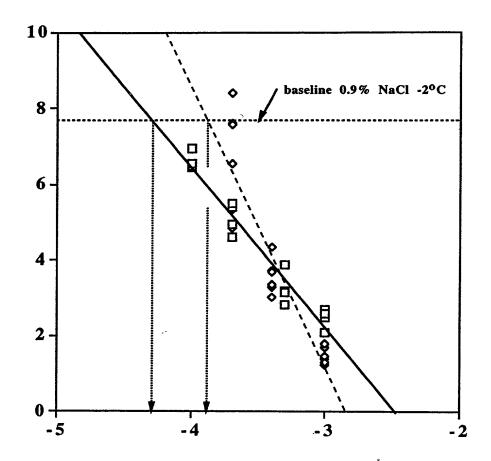


Fig. 8.28





log(dilution)

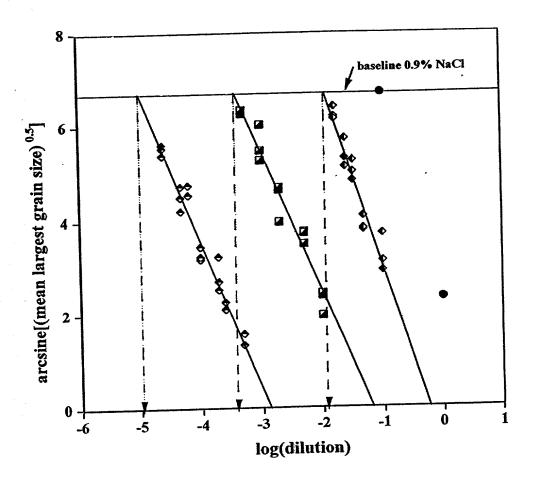


Fig. 8.30

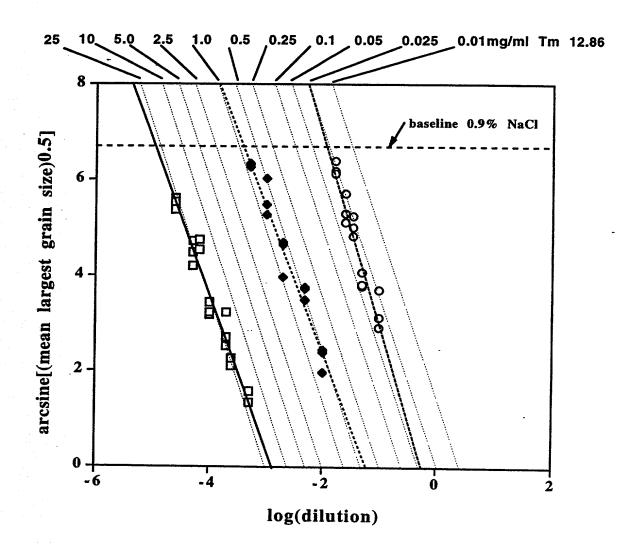
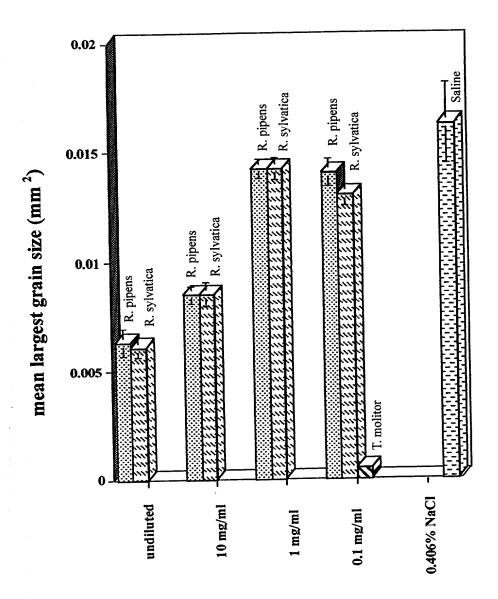


Fig. 8.31



dilution

Fig. 8.32

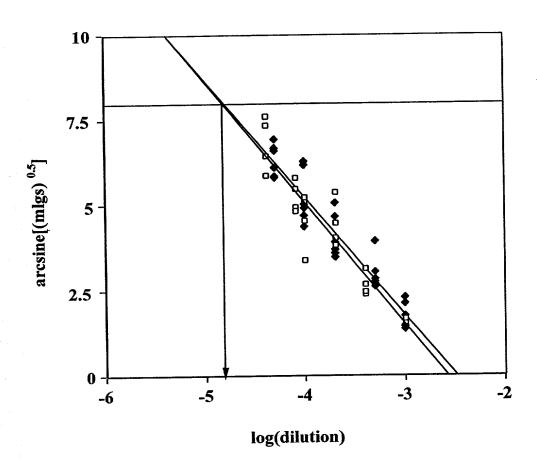
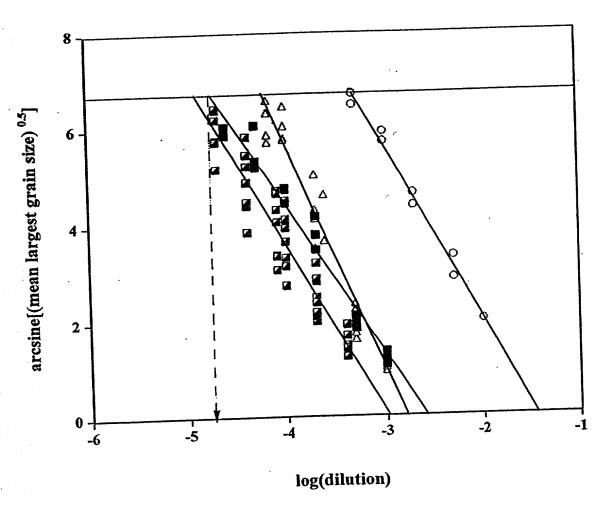


Fig. 8.33



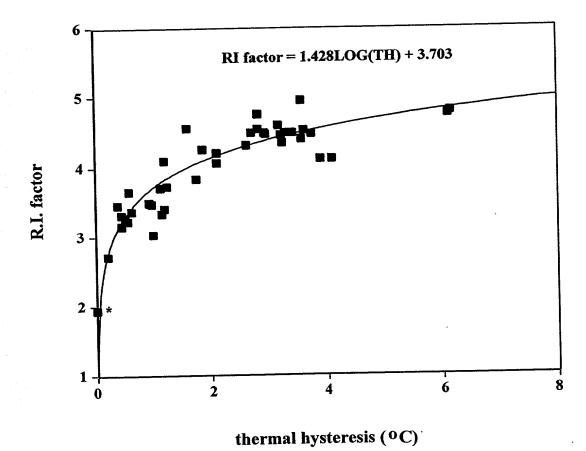
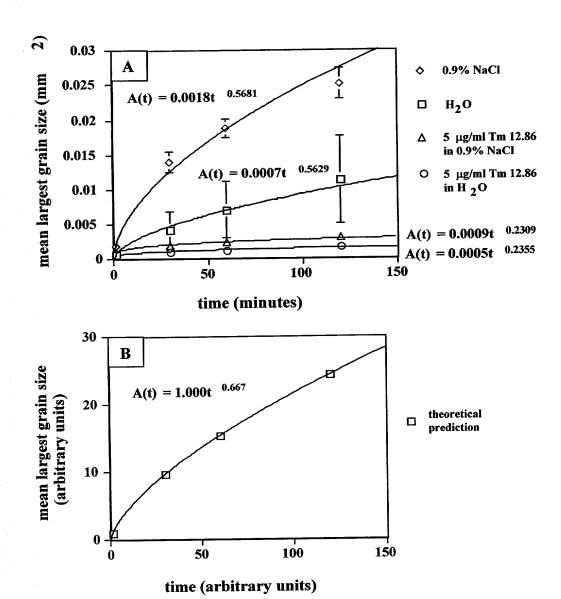
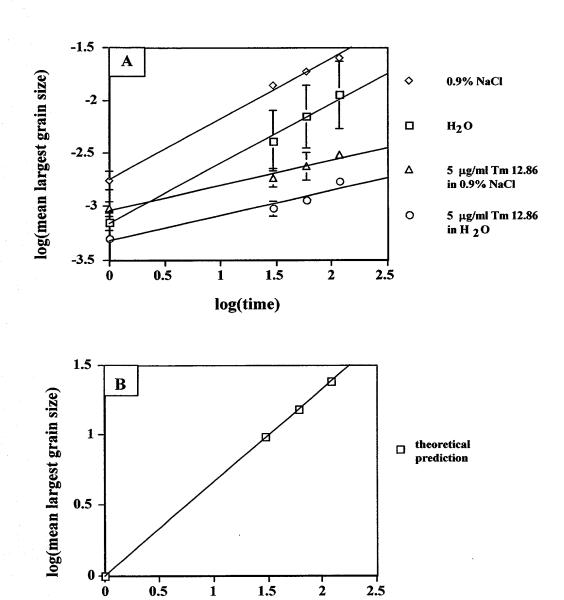


Fig. 8.35





log(time)

"Sandwich" method of R.I. assessment

1.

10 mm diameter cover glass

liquid samples (~0.1-0.2μ)





4. PLACE ON COLD STAGE, ANNEAL AT -6. C UP TO 12+ HOURS

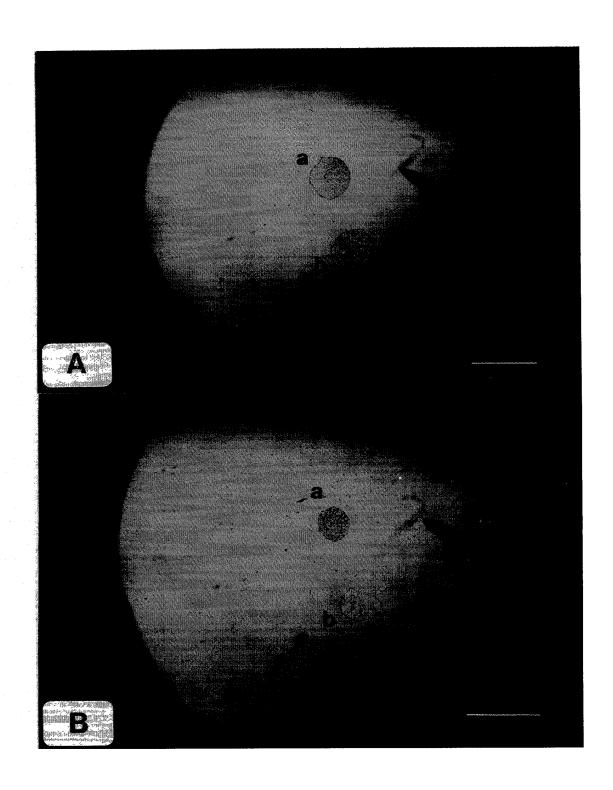


Fig. 8.39

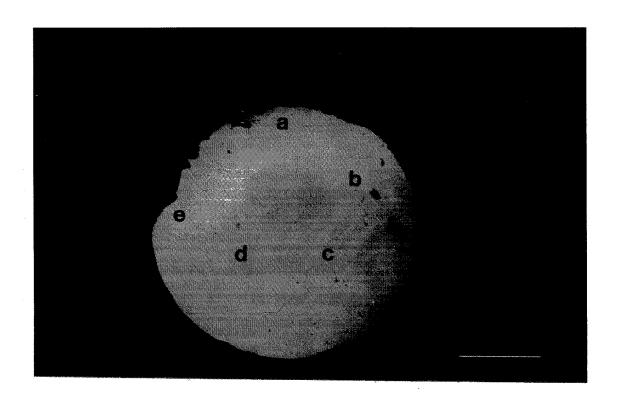


Fig. 8.40

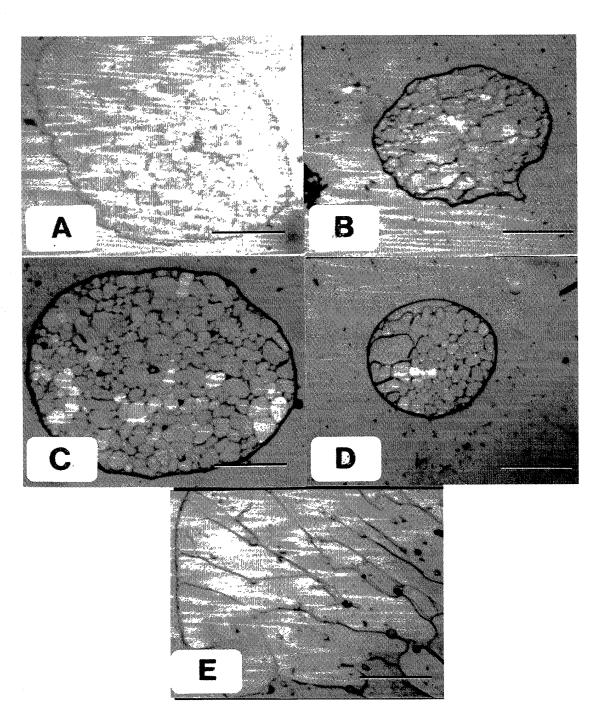
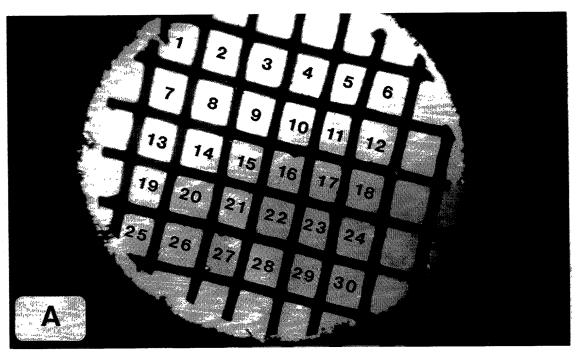


Fig. 8.41



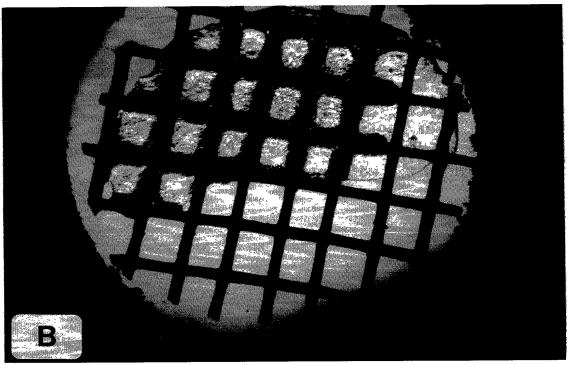


Fig. 8.42

DNA sequence of Tm 13.17 cDNA clone

	В			E																
	a			C																
•	m			0																
	H			R																
	I			I																
1	AGTG	GAT	CCA	AAG	AAT	TCG	GCA	CGA	GAC	TAC	TAP	CAT	GAA							
												M	K	<u>L</u>	<u>L</u>	<u></u>	<u></u>	<u>L</u>	<u> I</u>	<u>s</u>
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	<u>L</u>	I	L	L	V	T	V	Q	<u>A</u>	AL.	T	B	A	Q	I	E	K	L	N	K
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121	AGAT	CAG	CAA	AAA	ATG	TCA	AAA	TGA	AAC	FIG.	AGT	rgro	GCA	AGA	GAT	CAT	AAC	CAA	AGC	TC
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181	GCAA	CGG	TGA	CTG	GGA	GGA	CGA	TCC	TAI	\AC'I	'GAI	AACG	CCA	AGT	TTT	TTG	CGT	GGC	CAG	GA
	N	G	D	W	E	D	D	P	K	L	K	R	Q	V	\mathbf{F}	C	V	A	R	N
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241	ACGC	CGG	arCa	'GGC	CAC	GGA	ATC	GGG	3AG	AGGT	rGG?	rggi	CGA	CGI	GTT	GAG	IGGA	GAA	GGT	GA
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poly (A) tail